COOPERATIVE BREEDING HELPS BROWN-HEADED NUTHATCHES (*SITTA PUSILLA*) FACE INTERSPECIFIC COMPETITION

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ABSTRACT

Cooperative breeding helps Brown-headed nuthatches (*Sitta pusilla*) face interspecific competition, Esther Leah Niemasik, Ph.D. Cornell University 2019

My dissertation research tests the previously untested idea that heterospecific competition functions as an important selective force on group size in cooperatively breeding birds, using experimental approaches with the Brown-headed nuthatch (BHNU) (*Sitta pusilla*). We know from prior work that the western bluebird, which is much larger, excludes nuthatches from nesting sites (Stanback 2011). I tested the following explicit hypotheses: Hypothesis 1: Inter-specific competition selects for increased group size (more individuals helping or co-breeding) in BHNU and may even be sufficient to select for helping. Hypothesis 2: BHNU groups collectively defend against nest competitors, and collectively monitor their nest sites to chase away intruders.

I studied the effect of interspecific competition on the fitness consequences of cooperative breeding using an experimental design that involved creating high and low competition sites on golf courses. Half of each course was randomly assigned to the high and low heterospecific competition treatments; high competition territories had a single nest box whereas low competition territories had a pair of identical boxes within 5 m of each other at each box location. If competition acts as a selective force, reproductive success should be lower under high competition. And this is indeed what I found. GLMMs and AICc model selection criteria helped determine the model that best fit the data. The most highly supported model included both competition pressure and group size (ΔAICc >2), strongly supporting hypothesis 1. More BHNU nests failed on high competition sites (N=156, GLMM, course as fixed factor, p=0.043). Larger groups more often fledged young regardless of competition pressure, but small

groups were significantly less likely to fledge young in the high than low competition treatment and this higher failure rate was due to competition. I examined the behavior of BHNUs facing competitors using simulated territorial intrusions and nest watches. Larger groups were less likely to have intruders investigate their nest site without their knowledge and retaliation (GLMM, box as fixed factor, n=202, p=0.044). Furthermore, it took large groups less time to chase intruders from their territories. The mechanisms through which helpers increase breeder success in the face of heterospecific competition are twofold, first, they provide more noise and mobbing effort, and secondly they provide more eyes on the nest site, such that competitors are spotted more quickly. Cooperative breeding rescues BHNUs from the omnipresent pressure of competitors and provides a hitherto unconsidered benefit of breeding in groups.

BIOGRAPHICAL SKETCH

Esther Niemasik, born Esther Cline, was raised in Missouri next to a farm. There she developed an early fascination with the natural world. From capturing minnows in the local creek, to learning the names of all the birds that frequented their backyard feeders, she was a naturalist from a young age. Once old enough to fledge, she journeyed east, to North Carolina to attend undergraduate school at Davidson College, earning a double Major MA, in Biology and English. While there, she embarked on her career as a field biologist, learning some of the techniques that would serve her well in graduate school. After college she spent a year studying Florida Scrub Jays before beginning the graduate school application process. Cornell was her home for the next six years, culminating in this dissertation on Cooperative Breeding in Brown-headed nuthatches.

Dedicated to my husband, who has endless patience; and to my family who encouraged me to love nature.

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TABLE OF CONTENTS

CHAPTER 1

Cooperative breeders: better at interspecific competition?

Cooperative breeding can provide indirect and direct benefits to offset at least some of the costs imposed by not breeding independently. One such benefit, increased ability to compete with heterospecifics for access to limiting resources, has been underappreciated as a driver of social behavior. If collective defense against heterospecifics provides an important selective force for cooperative breeding, several key predictions must be met: 1) Cooperative groups should form more often around scarce and shared resources. 2) Group defense should focus on heterospecific competitors. 3) Larger groups should be more successful in heterospecific defense. 4) Cooperative defense should occur more frequently in groups of relatives. Non-relatives may form temporary cooperative associations when the resource is divisible, or external competitive constraints are particularly strong, but these associations will be very vulnerable to dissolution when external pressures decrease. I will address the evidence supporting each of these predictions and then discuss the ways competition among species can structure communities by defining niche spaces through degrees of sociality, i.e. more social species occupy different niches than less social species. Finally, I call for more experimental studies on the connections between heterospecific competition and cooperative breeding, particularly in the vertebrate world.

Keywords: Cooperative breeding, heterospecific competition, fortress defense

Highlights:

- Heterospecific competition can drive the evolution of cooperative breeding.
- Examples in many taxa suggest a causal link between competition and cooperation.

- When species suffer interference competition cooperation is especially impactful.
- Fitness return of cooperatively facing heterospecific competition is under-researched.

Introduction

Cooperative breeding, when more than two individuals cooperate to raise young, has elicited significant research to explore the potential for indirect and direct benefits to offset the costs imposed by not breeding independently (Koenig and Dickinson 2004, Riehl 2013, Stacey and Koenig 1990). This review argues that one such benefit, the increased ability to compete with heterospecifics for access to limiting resources, has been underappreciated as a driver of social behavior. A range of studies in disparate taxa support the idea that inter-specific competition provides benefits for breeding in cooperative groups when in competition with other species over key resources (bacteria- Celiker and Gore 2012, insects- Korb and Foster 2010, Sun et al. 2013, arthropods- Duffy et al. 2002, Purcell and Aviles 2012, fish- Taborsky and Limberger 1981) . Students of competition theory might here object that regardless of the potential of heterospecific competition to drive cooperation, all cooperative individuals are facing competition with others of their own species much more frequently (as they are necessarily encountering them on a daily basis) than they will face heterospecific competitors, and as intraspecific resource requirements always completely overlap the strongest competition any species will face is intraspecific (Nicholson 1954; May et al. 1974). Despite these facts heterospecific competition can still drive the evolution of cooperation if competition with intraspecifics is much less costly (either in resources lost or in damages received) than competition with heterospecifics. Heterospecifics may also carry advantages in competition that require increased levels of cooperation to overcome, as when a kleptoparasitic thrips attacks with

a venomous sting and it takes many cooperative thrips soldiers to immobilize it (Chapman et al. 2002, Crespi and Mound 1997, Choe and Crespi 1997). In this review, I show how the benefits of group-defense against heterospecific competitors can provide a major selective context for increasing the extent of cooperation, even driving some individuals to give up personal reproduction to help family members retain access to resources required to breed.

What does mathematical modeling have to say?

Several recent theoretical models suggest that interspecific competition should increase cooperation within groups or species. Reeve and Holldobler (2007) modeled competition among social insect colonies over a shared resource, and found that the tug of war between groups promoted cooperation within a colony, while decreasing investment in detrimental competition for resources within colonies. As they point out, there is no mathematical reason these groups must be of the same species for selection to favor increased cooperation. On a grander scale, researchers modeling competition between species found that species that invest more resources in wasteful intraspecific competition, which increases the productivity of individuals, but decreases the productivity of a population as a whole, had a higher risk of extinction than species that did not invest heavily in intraspecific competition (Rankin et al. 2007). Thus the frequency of cooperative species within species assemblages increased over time (Rankin et al. 2007). Conversely, Courchamp, Grenfell and Clutton-Brock (2000), modeling obligate cooperative breeders in interactions with competitors, predators, or parasites, found that cooperative species had a higher risk of extinction than non-cooperative species. Their model, unlike the other models, explicitly gave cooperative species an inverse density-dependent structure, such that smaller groups had poorer reproductive output (Courchamp et al. 2000). Thus interactions with other species frequently drove cooperative species below the critical group size and put their

populations into perpetual decline and eventual extinction through Allee effects, which are positive relationships between conspecific densities and individual fitness (Courchamp et al. 2000, Stephens et al. 1999). Modeling suggests that interspecific competition can favor cooperation, while also being disproportionately harmful to cooperative species due to small effective population sizes.

Growing experimental evidence supports the idea that intergroup conflict increases intragroup cooperation (Radford 2008, Radford 2011, Bowles 2009, Crewfort 2013, Poilizi et al. 2012, Puurtinen and Mappes 2009, Radford and Fawcett 2014, but see di Sorrentino et al. 2012). These studies focus primarily on conflicts between group living primates (including humans) with a few forays into the interactions of group living birds. Although most studies found a connection between inter-group conflict and increased levels of competition, most have difficulty directly connecting these increases to fitness gains or losses (Radford and Fawcett 2014, Radford 2008, Radford 2011), with the exception of the wonderfully executed study on capuchin monkeys that linked lost contests with increased movements, increased energy expenditure and decreased fitness (Croofort 2013). Because many of these results are based on behavior, rather than fitness measures, they provide only a weak test of the hypothesis that between-group conflict increases costly and meaningful cooperation. Moreover, this review focuses on the role of conflicts between species at limiting resources to promote cooperation, rather than on intraspecific contests between groups. The ability of intra-group conflicts to increase within group cooperation provides an important proof of concept, but such conflicts and their relationship to cooperation have received comparably much more research than heterospecific competition and the role it can play.

What are the key predictions of this hypothesis?

The hypothesis that collective defense against heterospecifics provides an important selective force for cooperative breeding makes several critical predictions: 1) Cooperative groups should form more often around scarce and shared resources. Resources with a clumped distribution in space will be particularly vulnerable to this effect, as a single cooperative group could defend the entire local resource and increase their marginal fitness gain much more significantly over non-cooperators than they would be able to if the resources was dispersed in distribution. 2) Group defense should focus on heterospecific competitors. 3) Larger groups should be more successful in heterospecific defense. 4) Groups of relatives should be more likely to cooperate in defense as they gain both indirect and direct benefits. I will address the evidence supporting each of these predictions in dyadic interactions between species, and conclude this section with a discussion of the potential advantages of increased sociality in both interference and exploitative competition Then I'll discuss the ways competition among groups of species influences the degree of cooperation within individual species and impacts the community of species that can overlap in space. I will conclude with a list of caveats and a call for more exploration of interspecific competition as an important driver of social behavior.

Are scarce shared resources more likely to promote cooperative breeding?

On the African savannah both currently, and in prehistory, large carnivores abound (Rose and Marshall 1996). As all require similar resources, and quite large amounts of these resources, competition among carnivores strongly affects carnivore abundance and distribution (Watts and Holekamp 2009). For example, lions frequently lose kills to hyenas and vice versa (Carbone, Du Toit, and Gordon 1997). Rose and Marshall (1996) hypothesize that competition between carnivore species accounts for the high levels of sociality found in predators on the African savannah (Rose and Marshall 1996). Three of the five main predators live exclusively in large

cooperative groups; lions, (*Panthrea leo*), spotted hyenas (*Crocuta crocuta*), and Africa wild dogs (*Lycaon pictus*), while another lives in small cooperative groups (cheetah, *Acinonyx jubatus*), and only the leopard (*Panthera pardus*) is truly solitary. Overall, sociality appears to be a more competitive strategy than hunting solitarily (Mosser and Packer 2009), and most large predators on the savannah adopt it. Early hominids also had a penchant for scavenging meat on the African savannah (Rose and Marshall 1996). Some anthropologists hypothesize that competition with other large social predators on the prehistoric savannah drove early hominids to increase their own levels of sociality and cognition (Rose and Marshall 1996). Early humans cooperated to drive off other competitors and to carry their prey to a more defensible site, where it could be broken down and consumed in relative safety (Rose and Marshall 1996).

For bacteria infecting other organisms iron frequently limits productivity (Harrison et al. 2008). Cooperative strategies for harvesting and fixing iron are relatively common in bacterial species; however, all such cooperators are vulnerable to "cheats"- cells that don't perform the expensive cooperative behavior that increases iron availability in the environment, rather they simply uptake the increased iron (Harrison et al. 2008). The bacterium that causes cystic fibrosis (*Pseudomonas aeruginosa*) faces this dilemma. Researchers have found that the presence of a heterospecific competitor, even one *P. aeruginosa* could dominate caused an increase in cooperative iron production (Harrison et al. 2008). This increased cooperation, however, also increased selection for cheats and incidences of cheating in the population also increased in the presence of the competitor (Harrison et al. 2008).

Another potentially contentious but a necessary resource is the nest cavity. Many species in various phyla nest in cavities and a suitable cavity is essential to reproduction for these species. However, suitable cavities are temporally and spatially rare resources (Cockle, Martin

and Wesolowski 2011, Lindenmayer et al. 2012, Martin, Aitken and Weibe 2004). If interspecific competition figures importantly in the evolution or maintenance of cooperative breeding then nest cavities would be a likely place for this to occur. And cooperative breeding, at least for African species, is more common in cavity nesting birds than in birds using any other nesting substrate (Du Plessis et al. 1995).

Snapping shrimp, *Synalpheus spp.*, also live inside defensible, rare resources, the canals of sponges in the tropical waters of the Caribbean (Hultgren and Duffy 2012). Snapping shrimp species compete directly over host sponges with their powerful snapping claw (Hultgren and Duffy 2012). Although different species can co-occur in the same host sponge, they avoid doing so, even though nearly 100% of sponges are occupied (Hultgren and Duffy 2012, Macdonald et al. 2006). Eusocial or subsocial shrimp species occupy more sponges than solitary species and are less likely than solitary species to share their sponge with another shrimp species (Macdonald et al. 2006). Finally, African cichlids (*Lamprologus brichardi* and *Neolamprologus pulcher*), the only known cooperatively breeding fish, also live and breed in defensible, rare resources. In Lake Tanganyika, they breed in family groups and cooperatively defend shallow depressions under rocks in which they lay their eggs, raise their fry, and hide from predators (Taborsky and Limberger 1981, Wong and Balshine 2011, Wong et al. 2012). These depressions are rare, difficult to retain, and require much excavation and defense against both conspecifics and heterospecifics (Taborshy and Limberger 1981, Wong and Balshine 2011, Wong et al. 2012).

Nectar resources, which flowers must renew daily and are temporally and spatially unpredictable and high in energy content, are a frequent limiting resource for bee species (Lichtenberg, Imperatriz-Fonseca, and Nieh 2010). Several tropical stingless bee species have evolved to not only live in large eusocial societies, but also to forage in large groups

(Lichtenberg, Imperatriz-Fonseca, and Nieh 2010). These social foraging species are able to aggressively exclude less social foragers from flower patches in direct interference competition and they recruit more quickly to new flower patches and retain access to patches longer than species that forage solitarily (Lichtenberg, Imperatriz-Fonseca, and Nieh 2010). For stingless bees cooperative social foraging increases their competitive dominance.

In insects, one of the primary hypotheses for explaining cooperative breeding and eusociality is called fortress defense (Choe and Crespi 1997). Fortress defense suggests that sociality developed in species that both live in, and eat of, a large resource that is essential to survival and reproductive success. To the extent that such a resource cannot be effectively defended alone, collective defense becomes obligate, and, over time, this can lead to defensive specialization, such as in the highly specialized soldier classes we find in many eusocial insects today. Ant, termite, and even aphid soldiers have morphological specializations that include shield-like heads, venomous stingers, powerful jaws, kicking legs, and even "explosive" chemical arsenals (Choe and Crespi 1997, Wilson 1971). Fortress defense is frequently couched in the context of intraspecific competition and many species including paper wasps (Clouse 1995, Gamba 1978), clonal anemones (Ayre and Grosberg 1996, Ayre and Grosberg 2003), ants (Herbers 1986, Rissing and Pollock 1991), and bumblebees (Hogendoorn and Velthuis 1993) are more successful at repelling intruders when living in cooperative social systems. Similar nest site requirements, however, can drive even unrelated individuals to cooperate in the face of competition (Izzo et al. 2009), and thus the fortress defense hypothesis works equally well with heterospecific competition being the primary threat. Clonal trematodes (Kamiya and Poulin 2013, Leung and Poulin 2011), polyembryonic wasps (Harvey, Corley, and Strand 2000), social aphids (Abbot 2009), and social thrips (Chapman et al. 2002, Crespi and Mound 1997) all fit into

this pattern with sociality evolving to deal with heterospecific competitors and arising primarily through the development of a soldier class with specialized and exaggerated defensive weaponry. Indeed, as mathematical models (Reeve and Holldobler 2007) and the presence of so much highly specialized weaponry among social species (Choe and Crespi 1997, Wilson 1971) suggests increased cooperation among a defending species can promote increased cooperation among the attacking species to penetrate the group defense and access valuable held resources. This could lead to ever increasing levels of cooperation and defensive specializations in both species- a co-evolutionary cooperative arms race. Such co-evolutionary races may explain the globally clumped distributions of cooperative species; for example social hunters are over-represented on the African savannah (Rose and Marshall 1996, Mosser and Packer 2009) and cooperative foraging is especially common in the South American tropics (Lichtenberg, Imperatriz-Fonseca, and Nieh 2010, Biesmeijer and Slaa 2006). Contests among species over resources can slowly increase the amount of cooperative specializations developed by both species, and act to stabilize cooperation over long periods; any decrease in cooperative specialization in one species without a concurrent decrease in the other species level of cooperation or defensive ability decreases the ability of the first species to access necessary resources and thus decreases individual fitness.

Cooperatively breeding birds across the globe are over-represented in tropical regions (Jetz and Rubenstein 2011, Russell et al. 2004, Russell 1989) and in lower latitudes topical bee guilds become increasingly dominated by species that not only live in large eusocial groups but also forage in cooperative social groups (Biesmeijer and Slaa 2006). The tropics have long presented a biodiversity puzzle and are overall the most speciose areas on the planet (Hawkins et al. 2008, Francis and Currie 2003). One hypothesis for increased tropical diversity is the

prevalence and increased importance of biotic interactions, like competition, in tropical regions (Schemskeet al. 2009). Although evidence remains mixed and more research is necessary, more and more phylogenetically controlled studies demonstrate increased biotic interaction pressures including predation, parasitism, and competition in low latitudes (Schemskeet al. 2009). Perhaps the extreme diversity of competitors and their long associations drove more species towards sociality, leading to the greater numbers of social species in tropical regions.

Do defensive behaviors target heterospecifics over conspecifics?

Social shrimp collectively defend their sponge hosts from heterospecific invaders using their enlarged snapping claw to direct coordinated, defensive displays at intruders, and eventually attack them (Duffy et al. 2002, Toth and Duffy 2005). They direct these displays preferentially towards heterospecific over conspecific intruders (Duffy et al. 2002). Similarly, cichlid helpers spend more time than breeders defending their nest caves from heterospecific intruders (Taborsky and Limberger 1981). While conspecific invaders also occur, these are primarily defended against by breeders (Taborsky and Limberger 1981), suggesting specialization of helpers for inter-specific defense. Groups of noisy miners (*Manorina melanocephala*) and bell miners (*M. melanophrys*) also aggressively expel all other organisms that enter their territories, including lizards, birds, and mammals (Dow 1977, Loyn et al. 1983). In the bell miner, interspecific territoriality increased the number of insects found on trees within their defended colonies, providing more food for group members (Loyn et al. 1983, Clarke and Schedvin 1999).

Gall dwelling thrips (*Kladothrips spps.*) present a prime example of how heterospecific defense can drive increases in sociality. Thrips live and feed entirely inside plant galls that foundresses create (Chapman et al. 2002, Crespi and Mound 1997), and they face constant

competition for these galls from a poisonous kleptoparasitic genus, *Koptothrips*. Soldiers increase the chance that a thrips clone will survive a kleptoparasitic takeover attempt (Chapman et al. 2002, Crespi and Mound 1997, Choe and Crespi 1997), and thrips soldiers have behaviors designed to neutralize the kleptoparasitic species' poison (Chapman et al. 2002, Crespi and Mound 1997, Choe and Crespi 1997). Without soldiers the kleptoparasite enters and kills the entire thrips clan, indicating that the soldiers are vital to clone survival (Chapman et al. 2002, Crespi and Mound 1997). Similarly, in aphids, gall dwelling species that suffer high levels of intra- and interspecific competition are more likely to evolve soldiers, and to have more highly developed soldiers the more competitive pressures they face (Abbot 2009).

Some of the best examples of the power of competitors to select for increased cooperation lie in the less classic cases of eusociality, namely polyembryonic wasps and parasitic trematodes (*Philophthalmus sp.).* Only parasitic trematodes that frequently share their host snail with other species have evolved a soldier class, and this species does better in admixtures than its soldier-less competitor (Leung and Poulin 2011). Moreover, when competitors are present trematode clones that possess soldiers have higher reproductive outputs than their soldier-less peers (Kamiya and Poulin 2013) and soldiers are more prevalent in snails of the size most commonly parasitized by the heterospecific competitor (Leung and Poulin 2011). Additionally, when forced to defend themselves trematode reproductive morphs suffer large decreases in reproductive output (Kamiya and Poulin 2013). Finally, in trematode species that face lower competition pressures with fewer sympatric species and lower incidences of co-occurrence in hosts the soldier morphology is less well developed, suggesting an evolutionary causal link between heterospecific competition pressures and soldier morphological development (Nielsen, Johansen, and Mouritsen 2014).

Soldiers and a parasitic lifestyle also co-occur in the polyembronic wasp (*Copidosoma floridanum*, Giron, Ross and Strand 2006). These wasps lay an egg inside a developing moth; the larvae hatch into a clonal mixture of soldiers (which do not reproduce) and future reproductives (Giron, Ross and Strand 2006). Soldiers develop in two time stages, first when intraspecific competitors are likely, and they destroy the burgeoning clones of competitive females; and second when interspecific competition increases (Giron, Ross and Strand 2006). These second stage soldiers are behaviorally distinct, though also infertile, and seem to direct attacks towards heterospecific competitors exclusively (Giron, Ross and Strand 2006). Additionally, clonal wasps adaptively increase the number of soldiers they produce when exposed to chemical cues (either from newly deposited eggs or the venom of the laying female) that indicate the presence of a heterospecific competitor (Harvey, Corley, and Strand 2006). Thus soldier production is intimately related to heterospecific threat level.

Co-breeding burying beetles also cooperatively direct aggression towards heterospecific competitors. When burying beetles compete with flies and other decomposers, groups appear advantageous (Scott 1994). Cooperative associations are more successful than pairs at removing fly larvae and less likely to completely fail due to competition with flies or other beetles (Scott 1994, Sun et al. 2014). More recently, experimental studies with the burying beetle *Nicrophorus nepalensis* demonstrated that cooperation is a facultative response to competition pressure. By manipulating the temperature of a carcass, Sun et al. (2014) were able to induce naturally arriving beetles to either cooperate with, or expel, newly arriving individuals, dependent on the likelihood of fly competition, as warmer carcasses were more likely to attract flies and more likely to induce cooperation.

Does success at thwarting heterospecific competitors increase with group size?

In competitions between lions and hyenas over carcasses groups of individuals are better at retaining access to their kills than are single individuals and larger groups are more successful in competition with heterospecifics than are smaller groups (Carbone, Du Toit, and Gordon 1997). Intraspecific competition for access to meat also increases with group size, however, so carnivores must balance interspecific competition pressures with intraspecific competition pressures when determining group size (Holekamp et al. 2003).

Co-breeding in burying beetles also illustrates advantages of larger cooperative group sizes in the face of interspecific competition. When competition is low, females frequently prepare carcasses for burial alone (Scott 1998). Biparental care provides a benefit when beetles compete with other beetle species over a carcass, particularly for smaller species (Scott 1998). When the competitive arena grows larger still (adding flies and other decomposers) larger groups retain carcasses more frequently (Scott 1994, Sun et al. 2014).

Cooperative breeding species occasionally also increase group sizes and their degree of cooperative behavior when they act as invasive species and face a new community of competitors (Chapman and Bourke 2001). Fire ants (*Linepithema humile* and *Solenopsis invicta*) in their native habitat have multi-queen colonies but these colonies are distinct and do not merge with other colonies. After arriving in the southeastern USA they became unicolonial, recognizing all others of their species as nest mates and failing to distinguish and attack them. Something about invading new habitats caused two separate species to increase their group sizes (Chapman and Bourke 2001). Unicoloniality has made these two invasive ant species, *S. invicta* and *L. humile*, extremely successful competitors, likely contributing to their ecological success. Although they coexist with other ant species in South America, in the USA they quickly drive other species to extinction (Chapman and Bourke 2001). Only similarly social species that also

possess a size advantage have been able to successfully resist the cooperative invasion tactics of unicolonial fire ants (Blight et al. 2010). Furthermore, some of the most successful invasive species on the planet are eusocial, including the fire ants (*Linepithema humile*, *Solenopsis invicta*, *Wasmannia auropunctata*), wasps and termites (*Vespula vulgaris*, *Coptotermes formosanus* (Chapman and Bourke 2001), and even the western honey bee (*Apis mellifera*). Not only are social species often incredibly successful as invaders, but the flexibility in their social system can allow them to increase group sizes when new resources or environments become available (Chapman and Bourke 2001). Of course, cooperative breeders may also face larger conservation threats than their non-cooperative kin, as they often have restrictive habitat requirements and frequently begin with smaller population sizes making them vulnerable to allee effects (Walters et al. 2004, Chapman and Bourke 2001, Courchamp et al. 2000). Much more research is necessary to illuminate the full connections between conservation concerns and cooperative breeding, either as conservation threats or as species of concern (Walters et al. 2004, Chapman and Bourke 2001).

Are related groups most likely to reap the benefits of cooperative defense?

Related individuals experience direct and indirect benefits of cooperating when faced with heterospecific competition. Indirect benefits through the increased reproductive output of their relatives and the direct benefit of increased access to a scarce resource. Even single celled organisms can experience this fitness increase through cooperation. Clonal yeast cells that create a cooperative resource are more prevalent in a mixed cooperator and non-cooperator population when grown with a bacterial competitor than in its absence, suggesting, at least in lab created populations, that competition can maintain a cooperative trait in groups of relatives (Celiker and Gore 2012). Of the species discussed in this review most cooperate only with close relatives,

including social shrimp (Toth and Duffy 2005), noisy and bell miners (Dow 1977, Loyn et al. 1983), lions (Mosser and Packer 2009) and African cichlids (Taborsky and Limberger 1981). Thrips (McLeish, Chapman and Crespi 2006), social spiders (Agnarsson, Aviles, and Maddison 2013), aphids (Abbot 2009), trematodes (Leung and Poulin 2011), and the parasitic wasp (Giron, Ross and Strand 2006) are all either clonal or highly inbred, increasing their relatedness values to cooperators even above that of a close relative. Hamilton's classic explanation for the evolution of cooperation requires relatedness (Hamilton 1964) and helping largely occurs among groups of relatives (Riehl 2013), so it is unsurprising that cooperative defense benefits also primarily accrue to groups of relatives.

Not all of the species in this review, however, cooperate only with relatives. Some burying beetle and ant species will breed cooperatively with non-relatives (Scott 1998, Izzo et al. 2009). Although nonrelatives cannot gain indirect fitness benefits by cooperating when competing with heterospecifics, they can gain direct fitness benefits, such that interspecific competition increases cooperation among non-relatives. Groups of non-relatives may form for the purpose of cooperative defense when the resource in question is divisible, but these associations will be temporary and dissolve when external competitive pressures decrease. As we have already discussed, groups of burying beetles will raise young collectively on large carcasses but not on small ones (Scott 1994, Scott 1998, Sun et al. 2014). Individual beetles leave the carcass separately and in the order of their genetic contribution to the brood with the dominant female staying longest with the young (Scott 1994). The best example of non-relative cooperative breeding being driven by external competition, however, occurs in ants. Ant foundresses facing competition with larger or superior competitors will collectively found a colony with non-relatives to retain access to preferred sites (Izzo et al. 2009, Cahan and Helms 2012). These

cooperative associations frequently break down after colonies grow large enough to maintain themselves in the face of competition without the second foundress; foundresses seamlessly transition into violent competition and battle to the death (Izzo et al. 2009, Cahan and Helms 2012). Their abrupt switch suggests the only benefit of cooperation was nest survival in the face of interspecific competition for cavities (Izzo et al. 2009). Across many taxa and in many different environmental contexts, cooperative breeding provides competitive benefits when organisms compete over rare resources.

Exploitative versus Interference competition

Exploitative competition occurs when species interact only through dual use of a resource and one species limits the population of the other by using up the shared resource. Interference competition occurs when species interact directly. One species limits the population of the other by aggressively excluding it from some resource through a direct confrontation. Cooperative species seem especially poised to reap the benefits of enhanced competitive abilities when the competitive arena is interference competition, as larger group numbers and defensive specializations will have the strongest effect in direct confrontations over resources. Of the species discussed in this review most demonstrate benefits of cooperating when faced with interference competition including lions (Carbone, Du Toit, and Gordon 1997), hyenas (Carbone, Du Toit, and Gordon 1997), stingless bees (Lichtenberg, Imperatriz-Fonseca, and Nieh 2010), cavity nesting cooperative breeding birds (Du Plessis et al. 1995), social shrimp (Hultgren and Duffy 2012), cichlids (Taborsky and Limberger 1981), social thrips (Chapman et al. 2002), social aphids (Abbot 2009), polyembryonic wasps (Harvey, Corley, and Strand 2006), trematodes (Leung and Poulin 2011), noisy and bell miners (Dow 1977, Loyn et al. 1983), and burying beetles (Scott 1994). A few species, however, either additionally or solely demonstrate

that cooperating can also be beneficial in the face of exploitative competition including yeast (Celiker and Gore 2012), *P. aeruginosa* (Harrison et al. 2008), stingless bees (Lichtenberg, Imperatriz-Fonseca, and Nieh 2010), and fire ants (Chapman and Bourke 2001). Overall, however, more species benefit from using cooperative breeding to master heterospecific interference competition and consequently commonly carry combat specializations (Wilson 1971).

Although many questions about the relationship between cooperative breeding and interspecific competition remain unanswered, I hope these sections have provided an overview of the evidence supporting cooperative defense against heterospecifics as a potential driver of cooperative breeding. Now I wish to take a step back and examine how competition between species, mediated by their degree of cooperation, might influence larger patterns of species distributions and community structures.

Can cooperation partition niches and ultimately create community structure?

Communities are inevitably made up of species that use resources differently, and lab experiments have shown that, at least for plants and bacteria, attempting to use the same resources in the same way will drive one of a species pair to extinction (Tilman 1977, Rothhaupt 1988, Grace and Wetzel 1998, Schoener 1983). While research on resource use has focused on both intrinsic differences in resource requirements and behavioral differences in how organisms acquire resources, researchers have paid little attention to the ways in which sociality itself can partition niches (MacArthur 1958, Tilman 1977). Mounting evidence suggests that such social niche partitioning occurs in communities inhabiting several different ecosystems (Purcell et al. 2012, Hultgren and Duffy 2012).

The genus of spiders, *Anelosimus,* in South America ranges in social behavior from nearly solitary, at one extreme, through subsocial (having maternal care) to communal, cooperative breeders at the other extreme with assemblages of species within a single patch of forest exhibiting a wide range in degree of sociality (Guevara et al. 2011). The most social species of spiders consistently capture larger prey than less social species (Guevara et al. 2011). Additionally, species that overlap in group size but differ in level of cooperation within groups also differ in prey capture sizes with the more cooperative species capturing larger prey. Prey sizes increase as the size of the colony increases (Guevara et al. 2011). Thus prey capture types are more over-dispersed than predicted by chance alone and degree of cooperation is the best predictor of prey capture sizes. Degree of sociality appears to partition niche spaces in these spiders. Levels of sociality also influence the microhabitat occupation patterns of this species group (Purcell et al. 2012, Purcell and Aviles 2008). Species with the largest colonies nest further into forest patches and in more central locations within the plants themselves, whereas species with smaller, more transient colonies, occupy forest edges and the extremities of plants (Purcell et al. 2012). Both habitat use and collective behaviors mediated by group sizes and cooperation partition niche spaces in this guild of spider species (Guevara et al. 2011, Purcell and Aviles 2008, Purcell et al. 2012).

Members of three genera of bees also appear to distribute themselves throughout the environment spatially, and temporally, by their degree of cooperation. In a study involving three bee species, solitary carpenter bees (*Xylocopa arizonensis*), subsocial bumblebees (*Bombus sonorous*), and the highly eusocial honeybee (*Apis meliflura*), Schaffer et al. (1979) examined how this bee community partitioned foraging space and time. All three bee species require nectar to feed themselves and their offspring. Solitary bees explore flowers in the smallest patches, and

at the least productive times of day (Schaffer et al. 1979). Nectar foraging is best in the early morning and early afternoon when flowers have had all night to refill nectar reserves, and when visited flowers have had time to refill their reserves throughout the day. Honeybees (the most social species) monopolize the most fruitful times of day and the richest flower patches (Schaffer et al. 1979). Bumblebees use the medium quality patches and times most frequently (Schaffer et al. 1979). As honeybees are smaller than bumblebees, levels of sociality appear to override body size differences in partitioning resource space. The less competitive solitary carpenter bees are the largest of the three, but they also have the lowest energy requirements and can thus persist even though highly competitive honeybees have forced them into low quality habitats (Schaffer et al. 1979). Again cooperation partitions a species guide in both space and time, suggesting that sociality can be a strong factor in building community structure.

Caveats to consider when researching interspecific competition and cooperative breeding

Here I have assembled evidence in support of the idea that heterospecific competition can select for increased within-species cooperation. Not all species, however, cooperate in the face of heterospecific competition, nor do those that do cooperate in all contexts. Davis et al. (2005) found that the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*) will not collectively defend roost cavities from interspecific invaders during the non-breeding season, even though they will defend nest cavities from the same competitors during the breeding season (Davis, Sieving and Kappes 2005). Seasonal shifts in the costs and benefits of cooperation in the face of competition may create complex and shifting strategies for organisms to maximize their relative fitness. It is therefore important to consider all potential costs and benefits of cooperating when examining interspecific competition as a driver of cooperative behavior.

A fascinating experimental manipulation of interspecific competition in termites demonstrates how the shifting costs and benefits of cooperating influence community structure and the competitive abilities of cooperative species. Researchers inserted colonies of two different species of termites into the same wood block in a lab setting and left them to co-exist for two years (Korb and Foster 2010). The species differed primarily in their response to the loss of reproductives (Korb and Foster 2010). One species fought viciously over reproductive vacancies, while the other allowed one of its workers to ascend without overt competition, or vicious fights (Korb and Foster 2010). When reproductives were left in the colonies both species co-existed in the wooden blocks without competitive exclusion (Korb and Foster 2010). However, when the researchers experimentally removed reproductives in both colonies prior to placing them together, nearly all of the colonies with vicious infighting over reproduction died out within the two-year experimental timeline (Korb and Foster 2010). Thus the more cooperative species had a strong advantage when in heterospecific competition, but species did not always differ in their levels of cooperation, as only the presence of a reproductive vacancy created competitive disparities and differences in cooperative behaviors.

Conclusions and recommendations for future research

Cooperative breeding provides many benefits to organisms from ants, to spiders, birds, mammals, and possibly even humans, when they interact with competitors over limiting resources. These benefits accrue to both relatives and occasionally non-relatives, and can have impacts on everything from individual battles over a limiting resource, to niche partitioning and creating community assemblages (Purcell et al. 2012, Macdonald et al. 2006). Perhaps we have underestimated the importance of community interactions, especially competition, when studying the benefits of coordinated social behavior, particularly cooperative breeding, where

group foraging and group defense of space and young operate throughout the life cycle and across seasons. We know that cooperative breeders are better able to repel predators when they mob cooperatively (Rabenold 1990), and better able to detect and expel brood parasites in large cooperative groups (Feeney et al. 2013), being able to expel heterospecific intruders more consistently aligns well with these known benefits. However, much of the evidence I have gathered here is correlational and distributional. In the future we need more experimental studies addressing the issue of competition in cooperative social systems. Only a few researchers have attempted to manipulate levels of competition to observe the response of cooperative groups (Sun et al. 2014, Korb and Foster 2010, Celiker and Gore 2012). No experimental study to date has addressed these issues in vertebrates. The evidence gathered in this review suggests that we need more such studies to illuminate the connection between competition and cooperative social behaviors.

Furthermore it remains unclear, if a relationship does exist between cooperative breeding and interspecific competition, whether the relationship is causal. I.e. can interspecific competition pressures drive the evolution of cooperative breeding or is interspecific defense only one of many benefits of cooperative breeding and some other factor, like intraspecific competition or climactic variability, drove the evolution of cooperative breeding (Emlen 1982, Jetz and Rubenstein 2011)? The immediate dissolution of groups of cooperative breeding non-relatives when external competitive constraints are removed suggests that, at least among non-relatives, interspecific competition can drive the evolution of temporary cooperation (Izzo et al. 2009, Sun et al. 2014).

Another area needing more research is the general importance of cooperative breeding in defining niche spaces and structuring communities. This review presents a couple examples of

sociality dictating space and resource use among communities of similar species, but how common is this relationship? Much more research is necessary to understand the role of social behavior in structuring communities. Overall, I recommend that researchers interested in social behaviors consider the whole community of species and their complex interactions and explore the possibility that interspecific competition could influence the costs and benefits of cooperation in their focal species.

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CHAPTER 2

COOPERATIVE BREEDING HELPS NUTHATCHES BEAT OUT HETEROSPECIFIC NEST COMPETITORS

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Abstract:

Understanding of cooperative breeding, where more than two individuals tend a single brood, is based mainly on examining competition within species with little attention paid to inter-specific competition. Experimentally reducing cavity competition with two other similarly sized bird species increased reproductive success in Brown-headed Nuthatches (*Sitta pusilla*), a species with 2 to 6 group members helping to feed at a single nest. These reproductive benefits included increases in the number of nest attempts, the probability of having a successful nest, and the per capita number of young fledged. Our findings indicate that failing to account for heterospecific competition may underestimate the fitness consequences of helping compared to independent breeding in cooperatively breeding birds.

One Sentence Summary: Competition with other species over resources can increase the benefits for helpers and breeders in cooperative groups.

Main Text:

Understanding of the costs and benefits of cooperative breeding, where three or more individuals tend a single brood, is primarily based on the exploration of the benefits of helping kin (kin selection theory) as well as ecological and social constraints on independent breeding, which are largely attributable to intra-specific competition, often for resources or mates (*1-4*). Recent research suggests that we have under-estimated the importance of inter-species interactions in determining the extent of cooperation within species (*5-7*). Rates of parasitism and predation have been linked to the evolution and maintenance of cooperative breeding (*5-8*). Accumulating evidence suggests that heterospecific competition can play a similar role. Pressures associated with heterospecific competition generate unusually high levels of cooperation both in novel laboratory created communities (*9*) and in un-manipulated populations in the field (*10*). No study to date, however, has experimentally tested the hypothesis that heterospecific competition generates per capita fitness payoffs for individuals that are sufficiently high to compensate for failing to breed independently. This would require both experimental manipulation of the intensity of inter-specific competition and measures of individual-based outcomes, such as per capita reproductive success.

We tackled this problem in a nest box population of Brown-headed nuthatches (*Sitta pusilla*), a small, philopatric, cooperatively breeding passerine native to southeastern pine forests

(*Pinus echinata* and *Pinus palustris)* in the southeastern U.S. Brown-headed nuthatches face intense competition over nest cavities with two similarly sized heterospecifics, Carolina chickadees, *Poecile carolinensis*, and House wrens, *Troglodytes aedon* (*11*). We predicted that: 1) individual reproductive success of Brown-headed nuthatches will be lower on high than low competition territories, 2) per capita reproductive success will increase with group size, and 3) there will be a significant interaction between the level of competitive pressure and group size, such that individuals in larger groups do not suffer the detrimental effects of heterospecific competition.

By creating an experimental system on relatively homogenous golf courses with sparse pine habitat, we were able to manipulate the availability of nest sites to create high and low competition sites without the interference that would result from variation in the number of suitable natural cavities. On low competition territories, resident groups had access to a *pair* of nest boxes suitable for Brown-headed nuthatches and other similarly sized birds. High competition territories had only one box. This created different levels of competition between high and low competition territories, which were distributed on 6 different golf courses (fig. S2). Brown-headed nuthatch nests were more likely to fail due to competition on high than low competition sites (GLMM, 0.58 ± 0.27, $z = 2.11$, $n = 290$ nest attempts, $p \leq 0.05$, fig. S2). The majority of these failures were due to usurpation by Carolina chickadees (49%) and House wrens (42%).

Group size had a significant effect on nesting success (i.e whether any young fledged): small groups had lower success rates than larger groups (GLMM, -1.60 ± 0.36, z value = -4.35, n = 276, $p \leq 0.001$, fig.1). Although this pattern held regardless of the competition treatment (high or low), groups that nested on low competition sites consistently had significantly higher success rates (based on having a nest that fledged at least one offspring) than groups that nested on high competition sites (GLMM, 0.57 ± 0.28, z value = 2.08, n = 276, $p \leq 0.05$). The difference in success rate was greatest for small groups, supporting our prediction that small groups would be more affected than large groups by competition (fig.1). Living in large groups insulated Brown-headed nuthatches from competition-related failures when competition pressures were high, and also increased success rates when competition pressures were low (fig.1).

The same patterns emerged when we examined a Brown-headed nuthatch group's probability of building a nest. Larger Brown-headed nuthatch groups were more likely to initiate nest-building (by filling cracks around nest box front with insulation material and placing ≥ 2.54 cm of nesting material in the base of the nest box) than smaller groups (GLMM, 1.14 ± 0.28, z = 4.06, n = 276, $p \leq 0.001$, fig. 2a). Groups, especially smaller groups, were less likely to initiate nest-building on high than low competition sites (GLMM, 0.68 ± 0.38, z = 1.72, n = 276, $p \leq 0.05$, fig. 2a). These patterns could be due to larger groups being more likely to contain older females (*12, 13*), however, groups did not differ significantly in breeding female age (ANOVA, F value = 1.97, df = 184, p = 0.10). Clutch sizes did not differ based on group size nor degree of competition (GLMM, 0.02 ± 0.03, z value = 0.86, n = 276, p = 0.39, fig. 2b). Eggs, although energetically costly, would represent a relatively low cost investment with a high fitness return in the event that competition is relaxed (*14*).

Larger groups also outperformed smaller groups in terms of annual reproductive success. The number of young fledged per season increased significantly with group size (GLMM, 0.19 ± 0.04, $z = 4.52$, $n = 276$, $p \leq 0.0001$, fig. 2c). Although there was a significant interaction between group size and level of competition (GLMM, 0.14 ± 0.06, $z = 2.32$, $n = 276$, $p = 0.02$, fig. 2c), contrary to our prediction, the disparity between high and low competition sites increased with group size. The number of fledglings produced per clutch was also significantly affected by an interaction between competition pressure and group size (GLMM, 0.20 ± 0.07, $z = 2.97$, $n = 276$, $p \leq 0.01$) with larger groups producing more fledglings under low competition pressure, but not when competition was high. We suspect that greater fledging numbers in larger groups occurred both because larger groups lost fewer individual eggs (more fledglings per clutch) and because they lost fewer whole clutches (more fledglings per season) to competitors like House wrens and Carolina chickadees. Additionally, as large groups retained possession of the nest box longer, they were able to double brood more often, producing more fledglings (fig. 2b, c).

Brown-headed nuthatches faced interspecific competition from two different species in a double whammy. Carolina chickadees and Brown-headed nuthatches (fig. 3a) are both resident species, and nest concurrently in North Carolina (*15*), tending to compete for nest cavities early in the season. Visible competition, involving replacement of nest material by Carolina chickadees delayed the first egg date of Brown-headed nuthatch groups by about 10 days (8.26 ± 2.18, $R^2 = 0.05$, t value = 3.79, df = 274, $p \leq 0.001$, fig. 4a). Groups with later first egg dates, including those whose laying was delayed by competition with Carolina chickadees, were more

likely to encounter House wrens (11.08 ± 1.96, $R^2 = 0.10$, t value = 5.65, df = 274, $p \leq 0.0001$, fig. 3b), which are migratory and return to North Carolina in mid-April (*16*). House wrens puncture and remove eggs and small nestlings of other birds to acquire a breeding cavity (*17*). They then fill the cavity with sticks, preventing other species from using it (*17*). Brown-headed nuthatch groups that encountered House wrens seeking nest cavities frequently lost their eggs or nestlings to House wren attacks. Larger groups, however, were less likely to lose eggs to House wren attacks (GLMM, 0.62 ± 0.30, z value = 2.05, n = 276, $p \leq 0.05$, fig. 3b), and more likely than smaller groups to retain possession of a cavity and re-nest following egg or nestling losses (GLMM, 1.82 ± 0.90, z value = 2.00, n = 57, $p \leq 0.05$, fig. 3c). Group size provided two direct defenses against the intrusions of House wrens. Large groups defended their eggs more successfully and groups of four were much more likely than smaller groups to successfully drive off the invading House wren and re-nest (fig. 3c). This is consistent with observations that collective vigilance is more likely to prevent the quick and furtive first visits of House wrens when more individuals are on watch (Niemasik, 2017, Chapter 3). Larger groups were also more likely to detect and prevent less overt forms of aggression, such as when House wrens attempted to fill the nest box with sticks. Larger group sizes provided benefits in heterospecific competition throughout the nesting season and these benefits were augmented in competition with a second competitor species, leading ultimately to increased annual fledging success for cooperative groups (fig. 2c).

While a group's reproductive success increased with group size, especially at high levels of inter-specific competition, this alone is insufficient to assert that heterospecific competition selects for increased group size in Brown-headed nuthatches. We therefore examined the effects

of group size and level of heterospecific competition on per capita fledging success for males and females. We analyzed males and females separately for two reasons. First extra-pair paternity rates are high (41%) and egg dumping rates are low (0%), leading to potentially different effects of group size on male and female fitness (*18*). Secondly most helpers (89%) were male, so the intraspecific competitive arena each sex faced was drastically different. Female per capita young fledged (defined as number fledged/number of females in the group) increased with group size with larger groups producing more young per female (GLMM, 0.15 ± 0.04, $z = 3.39$, $n = 276$ groups, $p \leq 0.001$); the interaction between level of competition and group size was also significant for females (GLMM, 0.16 ± 0.06, $z = 2.71$, $n = 276$ groups, $p \leq 0.01$), suggesting that larger groups insulate females from fitness losses due to heterospecific competition. Although group size also had a significant effect on male per capita fledging success (defined as number fledged/number of males in the group), the direction of the effect was opposite to that on females (GLMM, -0.27 ± 0.08, $z = 3.47$, $n = 276$ groups, $p \leq 0.001$). Group size negatively affected per capita fledging success of males and the interaction between level of competition and group size was not statistically significant (GLMM, 0.17 ± 0.09, $z = 1.77$, $n = 276$, $p = 0.08$). Despite the tendency for larger groups to suffer fewer fitness losses due to heterospecific competition, individual males did not experience greater per capita fledging success in large groups regardless of level of competition. This indicates that there was an intrinsic group benefit for females due to interspecific competition, but a fitness cost of increased group size for males, which suggests that males may face greater constraints on independent breeding than do females. These differences set up the possibility of conflict between males and females over group size.

Intensity of competition and group size both played important and interactive roles in determining which Brown-headed nuthatch groups initiated nest building, how many hatchlings were produced for a given clutch size, the overall probability that a Brown-headed nuthatch group would successfully produce young (fig. 1,2), and the per capita fledging success of both males and females. As suggested by Stanback et al. (*11*), competition with other species, even smaller species like House wrens and Carolina chickadees, limits Brown-headed nuthatch productivity (fig. 1,2). Larger group sizes, however, shelter Brown-headed nuthatches from heterospecific competition, benefitting females, and enabling groups to maintain possession of a nest box long enough to successfully fledge young (fig. 1). Brown-headed nuthatches are not alone in maintaining access to critical resources through group living; in social spiders (*Anelosimus spp*.) the most highly social species outcompete heterospecifics for choice sites at the forest core where insect richness is highest, and in social shrimp (*Synalpheus spp*.) the social and cooperative species are more prevalent and more likely to become the sole occupants of a host sponge than are solitary or subsocial species (*19, 20*).

Overall, as group size increased Brown-headed nuthatch success rates increased at two key levels: larger groups suffered less competitive exclusion when attempting to nest, and once they secured a nest they were less likely to lose it over the course of a season thus fledging more nestlings (fig. 1, 2). This highlights a larger pattern, that cooperative social behavior, and particularly increased group sizes, can insulate species from detrimental ecological interactions like predation, parasitism, and competition (*7, 21-25*). Heterospecific competition pressures represent a potentially overlooked agent of selection favoring cooperative breeding, and may be

a more important evolutionary driver of cooperation than previously considered, especially where critical and limited resources like nesting cavities are concerned.

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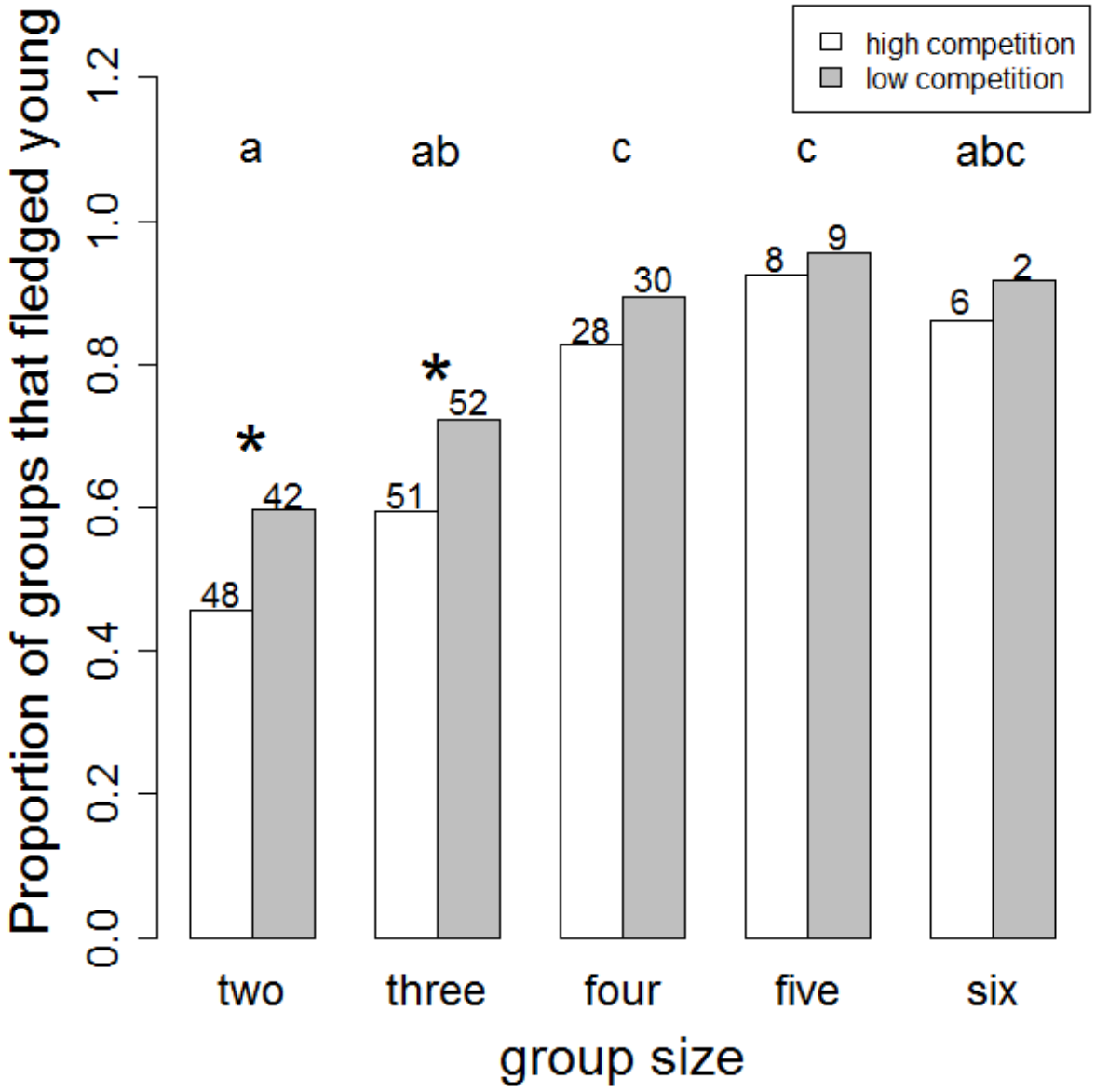

Fig. 1: Fitted proportion of groups fledging young as a function of group size for high and low competition sites. Bar pairs that do not share a letter are statistically different at α=0.05 level. Stars indicate statistically significant differences between high and low competition within a group size. Numbers written above the bar indicate the sample size at each group size.

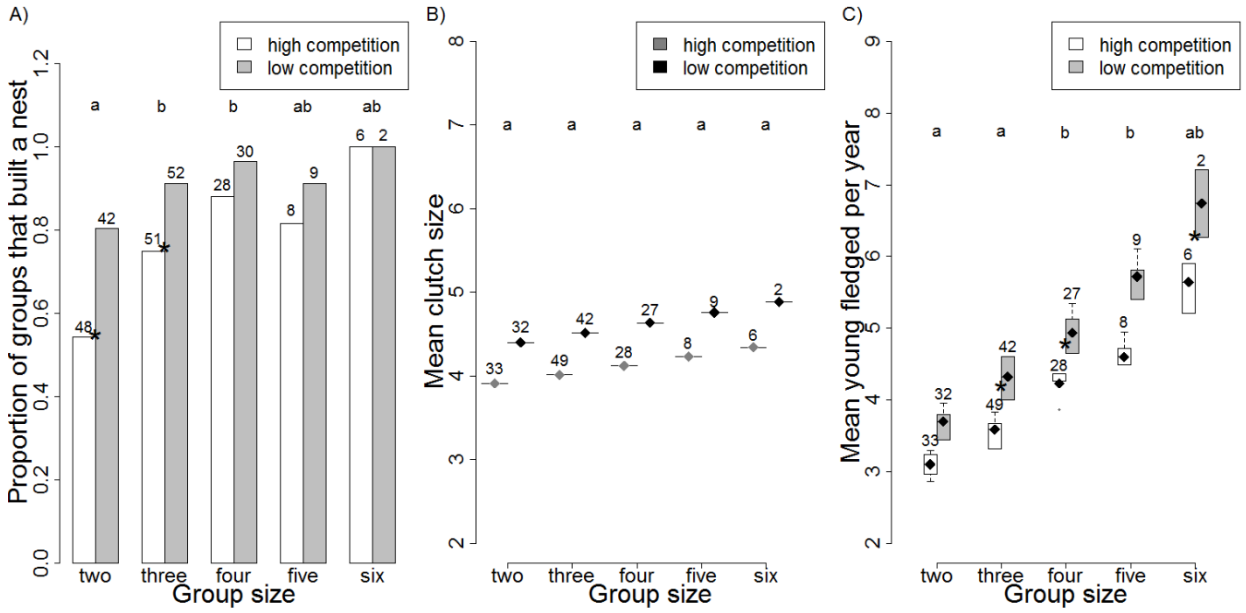

Fig. 2: Effect of level of competition on three components of reproductive success. Stars (*) indicate statistically significant differences between high and low competition. Numbers written above the bars indicate the sample size. Letters are used to signal statistical differences between group sizes, stars (*) indicate statistical differences between treatments within a given group size. Group sizes that do not share a letter are statistically different at α=0.05 level. Plotted values are fitted from the most supported model. **A)** Depicts nesting attempts. Best supported model included both level of competition and the size of the Brown-headed nuthatch group. **B)** Depicts clutch sizes. Best supported model included only level of competition. The first complete clutch was used for this analysis, even if the nest later failed. Only groups that attempted to nest were included in this analysis. **C)** Depicts annual fledglings produced. Best supported model included both level of competition and the size of the Brown-headed nuthatch group. Diamonds inside the boxplot indicate mean the number of young fledged annually for groups that attempted to nest.

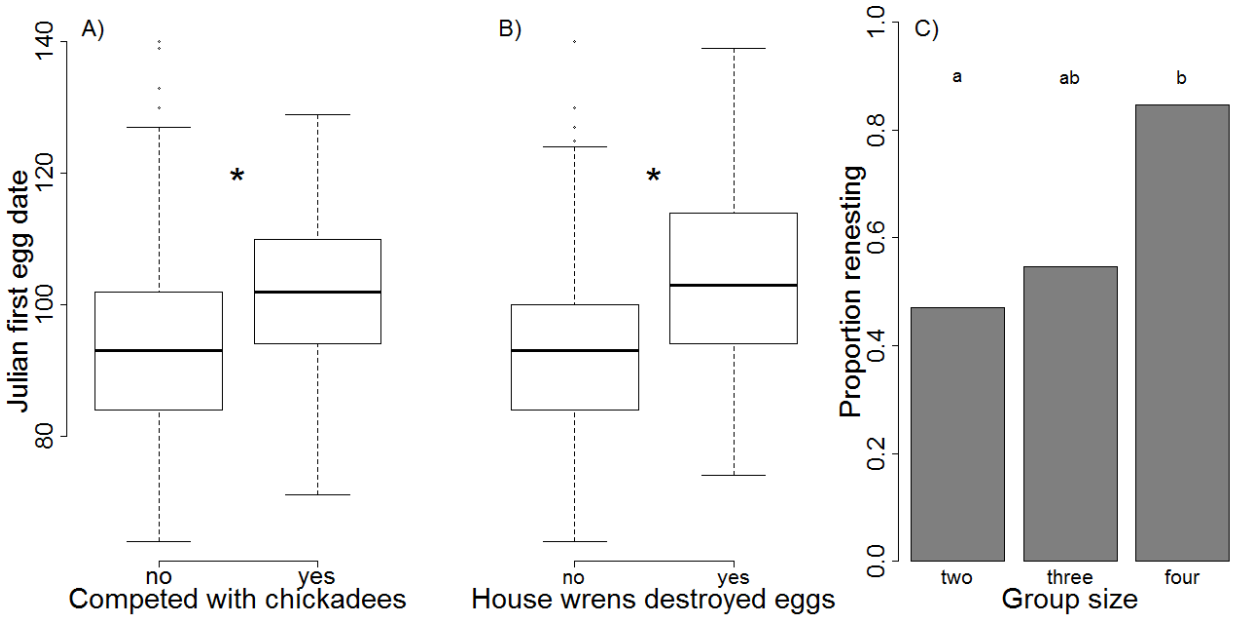

Fig. 3: Effect of competition on nesting date and probability of future competition. A) N = 276, Competition = direct interactions between Brown-headed nuthatches and Carolina chickadees at the nest. A star (*) indicates groups are statistically different at α=0.05 level. **B)** N = 276, House wrens were credited with egg losses when we found shells at the base of the nest box, sticks appeared in the box immediately following egg losses, or wrens began singing in the immediate vicinity shortly after egg losses. **C)** N = 52, Sample sizes for group of 5 and 6 were too small for analysis.

Table 1: Explanatory variables for the GLMM used to examine effect of competition and group size on three components of reproductive success.

Model Type	**Model Form**
Null	Dependent variable~(1|Golf course)
competition pressure	Dependent variable~level of competition + (1|Golf course)
group size	Dependent variable~group size + (1|Golf course)
competition pressure + group size	Dependent variable~level of competition + group size + (1|Golf course)
competition pressure * group size	Dependent variable~ level of competition * group size + (1|Golf course)

Table 2: Effects of level of competition and group size on two components of reproductive success based on model selection. The best supported model (**Δ**AICc < 2) included group size and level of competition. Both models used a binomial error distribution and contained field site as a random effect. AICc model-averaged parameter estimates (±SE). `P<0.10, *P < 0.05, **P < 0.01, and ***P <0.001.

	Probability of success		**Probability of nesting**	
Variable	**Model parameters**	**z score**	**Model parameters**	**z score**
Intercept	-1.85 ± 0.50	-3.6***	-1.48 ± 0.78	1.9`
Level of Competition	0.58 ± 0.27	2.1*	0.67 ± 0.39	1.7`
Group Size	0.76 ± 0.16	4.7***	0.99 ± 0.26	3.7***

Table 3: Effect of level of competition and group size on clutch size and annual number of young fledged in groups that laid eggs. Best supported models (ΔAICc < 2) included only level of competition as a predictor of clutch size and included both group size and level of competition as predictors of number of young fledged. All models used a Poisson error distribution and contained field site as a random effect. AICc model-averaged parameter estimates (±SE). `P<0.10, *P < 0.05, **P < 0.01, and ***P <0.001.

	Clutch size		**Annual N young fledged**	
Variable	**Model parameters**	**z score**	**Model parameters**	**z score**
Intercept	1.40 ± 0.05	30.1***	0.51 ± 0.15	3.3**
Level of Competition	0.11± 0.06	1.8`	-0.49± 0.23	2.1*
Group Size	NA	NA	-0.05 ± 0.05	-0.9
Group Size*Level of Competition	NA	NA	0.21 ± 0.07	3.1**

Supplementary Materials:

Materials and Methods

Figures: S1-S2

Tables: S1

References (*26*)

Supplementary Materials:

Materials and Methods:

We conducted this study on six golf courses located near Davidson, Mecklenburg Co., North Carolina, USA (35° 30’ N, 80° 50’ W) during the breeding seasons from February-Mid June in 2013 and 2014. Each course has approximately 25 box locations (max 41, min 15). Working on golf courses allowed us to control the number of cavities available to specific clusters of Brown-headed nuthatch territories. Because managers actively removed dead trees (normal nesting sites) on golf courses, we were able to standardize the number of cavities available for each year of the study. Golf courses consisted of stands of shortleaf pine (*Pinus echinata*) or pines mixed with common southeastern deciduous trees (*Cercis canadensis, Acer rubrum, Fagus grandifolia, Quercus alba, Quercus falcate, Liriodendron tulipifera, Liquidambar styraciflua, and Cornus florida*) separated by open fairway spaces. Understory was non-existent due to active management and a thick layer of pine straw. The open understory mimics southeastern pine forests immediately post-burn and is optimal for Brown-headed nuthatches (*26*). All nest boxes on the study site were equipped with small (26 mm) holes that excluded Eastern Bluebirds (*Sialia sialis*), and other larger competitors from entering. Excluding Eastern bluebirds provided opportunities for Brown-headed nuthatches to coexist with Carolina chickadees and House wrens, the two size matched competitors most likely to compete over natural nest sites. All nests were in Schwegler® “woodcrete” nestboxes with an interior diameter of 12 cm, mounted 1.75 m high on aluminum poles with ERVA® stovepipe-style predator guards. Boxes were separated by an average of 300 m, approximately the width of a Brown-headed nuthatch territory (*26*).

We monitored 138 groups of Brown-headed nuthatches on these six sites for two years. Groups of three were most common (37 %), followed by pairs (32%), groups of four (21%), and larger groups (10%). Most groups consisted of a breeding pair and their descendant male offspring (based on social pedigrees). Because of higher female mortality, groups periodically consisted of a group of related males and a single, unrelated, breeding female.

Adults were captured in mist nets or trapped in the nest box; nestlings were briefly removed from nests for banding (age 12-16 days). All individuals were given a unique band combination consisting of a metal USFWS band and three colored leg bands. A recording of a territorial call and a Brown-headed nuthatch model were used to encourage adults to fly into a mist net for capture. Territorial calls were played on a small handheld speaker and consisted of five mins of territorial song taken from the Macauley Library, Cornell Lab of Ornithology. The song was recorded in Florida. Models were set on a tree behind the mist net. Females rarely responded strongly enough to playback to be captured in mist nets, and were therefore ambushed on the nest with ping pong ball attached to fishing line, strung through the nest hole, and pulled to cover the opening when the desired individual entered the nest box. Captures on the nest were only performed after the nestlings were at least a week old, since females were prone to abandonment if distrubed prior to this age. Other adults continued to feed the nestlings as we waited for the targeted bird, so disturbance of feeding during capture was minimal.

Once we had the birds in hand, we took morphological measurements, a small blood sample ($\leq$ 25 ul), and banded them. Blood samples were collected from the bracheal vein of

adults and nestlings with a heparinized capillary tube, and stored in lysis buffer at a constant moderate temperature. Morphological measurements included tarsus (measured with calipers), wing chord, and mass. To take mass measurements Brown-headed nuthatches were placed in a small plastic bag and hung from a scale. Bags were punctured to allow free airflow, and no bird spent more than one min inside the bag. Banded adults were released onto their territories, and nestlings were returned to their nests.

In the first year of our experiment, we divided the two most and the two least productive golf courses in half, creating high competition conditions on one half of each course and low competition treatments on the other half. Golf courses are built with a natural half way point, since individuals frequently only wish to play nine, rather than eighteen holes. Thus the front nine provided an inherent contiguous separation from the back nine, as each extended out from, and returned to, the central clubhouse. We divided only these two courses to control for inherent site differences, since we knew a priori that these courses were more and less productive than all the others. In 2013, we assigned courses to one of two treatment groups: a *low* competition treatment, and a *high* competition treatment. Courses were assigned rather than randomized to assure that an equal number of box locations occurred in each treatment group. High or low competition treatments were then assigned to the groups of courses randomly. Since no birds shifted territories in 2013 in response to increased competition (see census section), in 2014 half of each course was randomly assigned to the *high* and *low* heterospecific competition treatments; high competition territories had a single nest box placed on them, whereas low competition territories had a pair of identical boxes within 5 m of each other at each box location.

Once a week in the first year of the study (2013), and once a month in the second year (2014), we censused all Brown-headed nuthatch groups in our study populations. All censuses were conducted between 0600-1000 am. Individuals were identified via their color band combinations. First, we searched territories; locating individuals either visually or aurally, using binoculars (Nikon 8 X 42) to acquire individual identities. To facilitate location and encourage individuals to descend low enough for easy identification, we frequently used a small handheld speaker to play a male Brown-headed nuthatch territorial call (described above) through an apple IPod mini. Calls were never played for more than five minutes and Brown-headed nuthatch groups showed no habituation to playback. Censuses were performed weekly in the first year out of concern that the competition pressure manipulation would cause groups to shift their territory boundaries, and possibly even fuse. Since shifts and fusion events were never observed in 2013 we reduced censusing effort to once a month in 2014. We observed 73 ± 2% and 80 ± 3% of group members per census in 2013 and 2014, respectively (Fisher exact, $p = 0.74$).

We visited nests once a week to record the species nesting, nest initiation date, failure dates, causes of nest failures, first egg date, clutch size, hatching success, death of adults (on nest), fledging success, and whether the nest was a first or second brood. The primary species on our field sites have very distinct nests (fig. S1), which can easily be visually distinguished. Carolina chickadees build primarily with moss, House wrens, with sticks, and Brown-headed nuthatches with shredded pine bark. We scored a nest as failing due to competition when nest materials shifted from one species to the other in the course of a week (fig. S1). Some nests were taken over multiple times over the course of a season (fig. S1). House wrens could cause nest failures without adding sticks, however, if the House wren only destroyed the eggs, or young, of

the nesting species. Thus we attributed failures to House wrens when we found shells at the base of the nest box, when sticks appeared in the box immediately following egg loss, or when wrens began singing in the immediate vicinity shortly after eggs disappeared. Because predator guards prevented egg losses to snakes and other predators, all egg disappearances could be attributed to competition. Failures were attributed to weather when eggs remained in the nest without hatching, or when dead nestlings were found in the nest. These events occurred mainly after extreme weather, either unusually cold or unusually hot for the time of year. We also recorded whether Brown-headed nuthatch groups chose to build a nest. Groups often built no nests but remained on their territories throughout the year and responded to playbacks. Territories were searched for natural cavity nests to confirm that no nest existed.

All statistical analyses were performed in R version 2.13.1, using the packages "lme4" and "AICcmodavg." We used generalized linear mixed models (GLMMs) with either a binomial error distribution (success, choice to build, and competition presence) or a Poisson error distribution (clutch size, and number fledged). Our sample unit was the group-year (n = 276). Groups were considered successful when they fledged at least one nestling during the season. Groups were scored as building a nest when they accumulated ≥ 2.54 cm of nest material in the bottom of a nest box, this accumulation indicated their willingness to initiate a nest, and allowed even groups that lost competitive battles early in the nesting process to be analyzed. All models included golf course (field site) as a random factor. For each explanatory variable we used model sets and ΔAICc to determine if group size and level of competition actually increased the fit of the model. Model sets all had the same form (Table 1), and ΔAICc values allowed us to select

the most supported model. Models with a ΔAICc <2 were averaged to produce the final best fit model. Significances reported in results are drawn from these best fit models.

Further analysis: Addressing the question of territory quality

Since only competition pressure was experimentally manipulated and group sizes were left to vary naturally, it is possible that differences in territory quality, rather than properties of the groups in the experimental treatment, were captured in these analyses. However, competition treatments were switched among sites between years, such that many territories experienced both levels of competition. As group size also naturally increased or decreased, we can ask whether naturally occurring changes in group size between years, while experimental competition pressure remained constant had the predicted effects on overall success; i.e. groups that increased in size were more likely to be successful, while groups that decreased in size would be less likely to be successful in the following year. Since these analyses required comparing probabilities of success in subsequent years, sample sizes were smaller than the analysis of fledging success, nesting probability, clutch size, and per capita fledge success, but χ^2 analysis revealed many of the same patterns as these analyses, with the impact of competition pressure being mediated by group size changes. Although the directional tendencies were not statistically significant, increases in group size had a smaller effect size than decreases in group size (Table S1).

Figure S1: Nest types used as evidence of competition. The bottom layer in this nest box is a Brown-headed nuthatch nest, primarily made of shredded pine bark. House wrens then took over the nest adding a layer of sticks. Carolina chickadees then took over from the House wrens, building their nest with moss and fur, and finally the House wrens took the nest back from Carolina chickadees, layering their sticks back on top. A simple picture shows the competitive history of this nest box, and the species battling to use it.

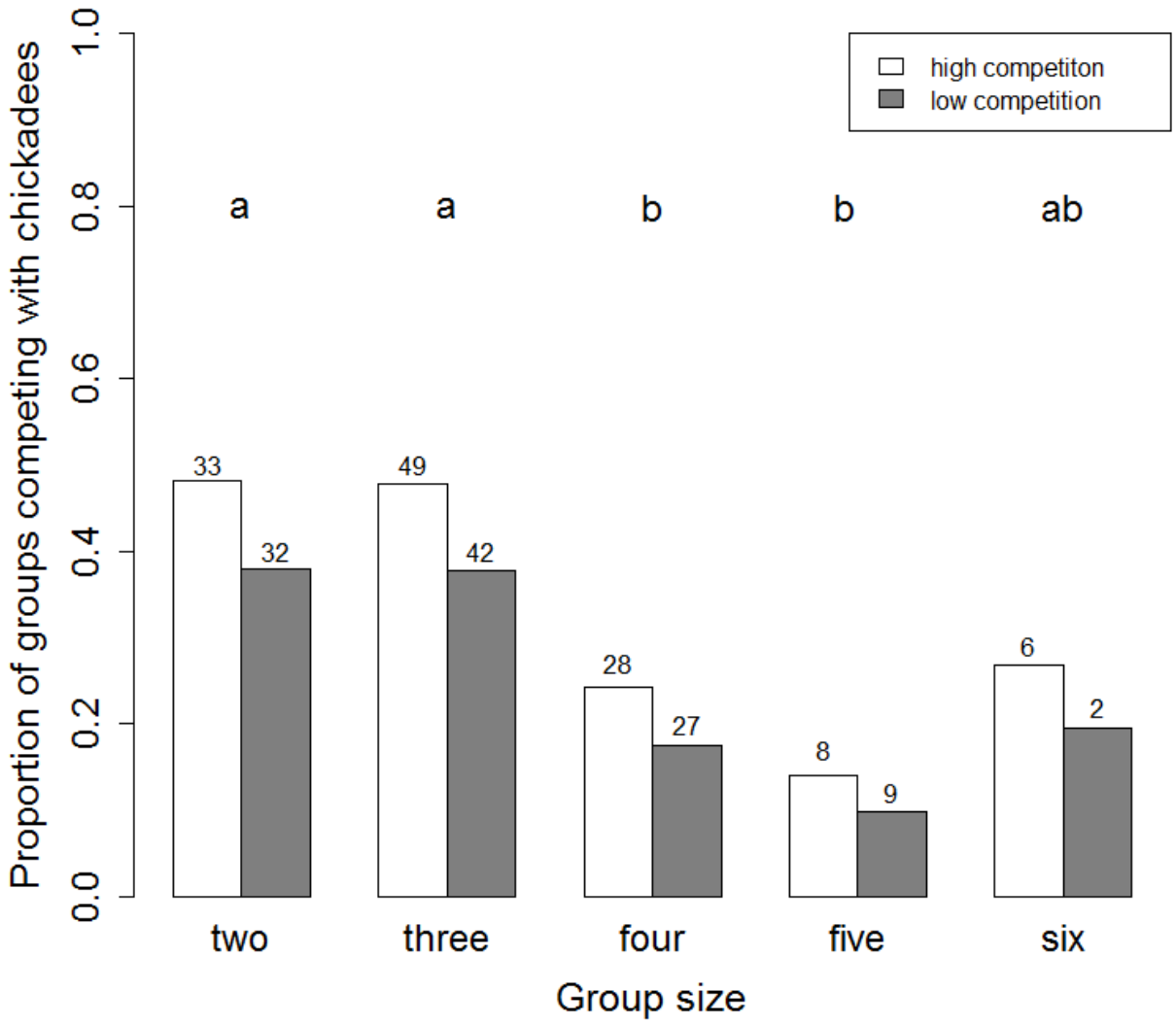

Figure S2: Effect of group size on the probability a Brown-headed nuthatch group would compete with Carolina chickadees. Plotted values are fitted from the most supported model, which included both competition pressures and Brown-headed nuthatch group size. Bar pairs that do not share a letter are statistically different at $\alpha=0.05$. Numbers above bars indicate sample size in group-years.

Table S1: Effects of level of competition and group size changes between years on probability of nesting success. Probabilities of success in year one were compared with probabilities of success in year two for each category. Successful groups fledged at least one nestling. Up arrows indicate increases in either level of competition or group size between years; down arrows indicate decreases, and equal signs indicate that the level of competition or group size did not change between years. All group sizes were lumped for these analyses, and groups were divided into small (2), medium (3) and large (4+), based on the asymptote of success, where groups of four five and six are equally successful.

Competition change (2013-2014)	**group size change (2013-2014)**	**Sample size**	**Prediction**	**χ^2 p value**	**Predicted direction?**
↑	↑	4	=	1	NA
↑	=	18	↓	0.53	no
↑	↓	10	↓	0.03	yes
=	↑	13	↑	0.83	no
=	=	30	=	0.3	NA
=	↓	18	↓	0.18	yes
↓	↑	5	↑	0.72	no
↓	=	12	↑	0.51	yes
↓	↓	12	=	0.77	NA

CHAPTER 3

COLLECTIVE DEFENSE AGAINST HETEROSPECIFIC COMPETITORS IN THE COOPERATIVELY BREEDING BROWN-HEADED NUTHATCH.

Abstract

The brown-headed nuthatch, *Sitta pusilla*, is a cooperative breeder whose fitness is strongly influenced by competition with similarly sized heterospecific competitors. Nesting success increases strongly with group size, an effect that can be diminished by reducing the intensity of interspecific competition. Models were used to test responses of group members to predators, competitors, and conspecifics. Models are good for measuring intrinsic responses to threat, because they remove variation in both individual and species-level aggression by the competitor or predator species. As such, they are effectively an assay for the degree to which brown-headed nuthatches perceive other species as a threat. We discovered that (1) brown-headed nuthatches use collective defense (group mobbing) equally against both competitors and predators, (2) larger brown-headed nuthatch groups had shorter latencies to response than smaller groups, and (3) at larger group sizes, a portion of group members dropped out of participating in defense. However, larger groups still had more individuals engaged in nest defense than did smaller groups. Collective defense in response to heterospecific competition appears to reduce the per capita costs associated with nest defense while simultaneously increasing the defensive capacity of brown-headed nuthatch groups.

Introduction

Cooperative breeding, when three or more individuals work together to raise young, occurs in nearly 9% of bird species globally (Cockburn 2006). Research on cooperative breeders has traditionally focused on the constraints imposed by intraspecific competition to explain why

some individuals give up personal reproduction (Emlen 1982). These extra individuals, known as helpers, frequently make the "best of a bad job" by raising kin. External constraints, like a limited number of suitable territories (e.g. Florida scrub-jay, *Aphelocoma coerulescens*) or the combination of a shortage of mates (e.g. superb fairy wren, *Malurus cyaneus*) and intraspecific competition for territories, prevent helpers from breeding independently (Pruett-Jones and Lewis 1990, Woolfenden and Fitzpatrick 1984). This view of cooperative breeding, while supported by substantial evidence, fails to consider the broader community of species within which cooperative breeders interact. Cooperative breeders are often territorial and some defend resources, like food supplies (acorns) (Koenig 1981) and nesting cavities (Walters et al. 1992, Walters et al. 2014), which other species also require to survive and reproduce. Cooperative defense against heterospecific competitors may be more common than is generally recognized and, if so, we should see: a) evidence of aggressive, collective defense against heterospecific competitors, b) evidence that success at detecting and evicting heterospecific competitors increases with group size, and c) evidence of increased resource holding potential of groups when faced with increased intensity of heterospecific competition. Niemasik et al. (2016) tested this latter prediction in brown-headed nuthatches (*Sitta sp.*), a cavity nesting cooperative breeder with group sizes of up to 8 birds (Chapter 2), showing that group size is associated with increased breeding success and that the effect of group size on fitness declined when heterospecific competition was experimentally reduced. This paper focuses on the first two questions, describing cooperative defense and examining how group size influences the success of a defensive bout.

Intraspecific competition has been examined extensively in cooperative breeders, primarily within the context of the benefits of dispersing in coalitions to take over new breeding vacancies (Hannon et al. 1985, Mosser and Packer 2009). Evidence also indicates that increased group size can confer benefits in defense against predators: large groups of chestnut crowned babblers (*Pomatostomus ruficeps*) are less likely to be attacked by a predator than are small groups (Sorato et al. 2012), and although they are not cooperative breeders, large groups of pied flycatchers (*Ficedula hypoleuca*) mob more intensely and effectively than small groups (Krams et al. 2009), which translates into higher nest survival (Robinson 1985, Brown and Lawes 2007, Krama et al. 2012, Sorato et al. 2012). Coalitions of defenders can be so valuable in rare, dangerous predation attempts that even species that don't live in groups, like the pied flycatcher, will mob cooperatively when pairs nest in close proximity, a form of cooperation that has been interpreted as reciprocity (Krams et al. 2009). If group defense is similarly important with regard to heterospecific competition, groups should defend cooperatively against other species of competitors, large groups should detect and respond to competitors more quickly than small groups, and large groups should evict competitors more readily.

Heterospecific competitors mobbed or attacked by groups, rather than individuals, will experience more attacks per unit time, and be more likely to be injured by the combined aggressive response. Heterospecific competitors can pose threats as large as those posed by predators, particularly in competition over a crucial, limited, breeding resource (Fisher and Weibe 2006). In cooperative cichlids 83% of defensive encounters were directed towards cavity competitors (both homo- and heterospecific), and only 17% towards predators (Taborsky and Limberger 1981); heterospecific competitors are also a more frequent threat than competitors. If

competitors are as aggressive as predators, mechanisms generally associated with predation can also be important in defense against competitors, including predator/competitor confusion (Garay 2009, Ioannou, Guttal and Couzin 2012, Olson et al. 2013, Ioannou et al. 2009), threat dilution (i.e. spreading the risk of heterospecific defense - injury or death - across more defenders) (Garay 2009, Olson et al. 2013, Ioannou et al. 2009), and an increased ability to detect predators/competitors (Sorato et al. 2012).

Collective defense against heterospecific competitors may be particularly important for cavity nesting species, because cavities are used by a large community of species in diverse phyla (Cockle, Martin and Wesolowski 2011). Cavity size and height requirements of different taxa frequently overlap (Blanc and Walters 2008, Fisher and Weibe 2006) and most terrestrial cavities occur in dead wood, which means that they often decline in usefulness over a short number of seasons, or even within the same season (Lindenmayer et al. 2012, Cockle, Martin and Wesolowski 2011). Intense competition occurs over these temporally rare and unpredictable resources (Newton 1994, Blanc and Walters 2008, Cockle, Martin and Wesolowski 2011). Cooperative breeders frequently breed in cavities (Du Plessis 1995, Walters et al 1992), and thus face contexts where collective defense carries high benefits.

Correlational research indicates that helpers can play important roles in heterospecific defense of cavities. Eusocial snapping shrimp (*Synalpheus regalis*) present a coordinated snapping display towards heterospecific intruders that enter cavities in their sponge homes with all individuals, but especially male helpers, directing more snaps towards heterospecific than conspecific competitors (Toth and Duffy 2005). Similarly, cichlid helpers (*Lamprologus brichardi*) spend

more time than breeders dealing with heterospecific intruders at their nest caves (Taborsky and Limberger 1981). Neither study, however, has considered the effect of group size on collective defense (Toth and Duffy 2005, Taborsky and Limberger 1981).

Brown-headed nuthatches are specialists on old growth pine forests and highly degraded snags, which are rare and ephemeral resources in a landscape maintained by frequent fires (Withgott and Smith 1998). They have variable group sizes ranging from 2 to 6 birds, and their reproductive success declines dramatically when they compete with eastern bluebirds (*Sialia sialis*), which often exclude them from nest cavities (Stanback et al. 2011). Old growth pine forests are communities rich in cavity competitors (Blanc and Walters 2008). Because brown-headed nuthatches are year-round territory holders with philopatric sons, they have limited access to cavities beyond the limits of their territories (Withgott and Smith 1998). They nest readily in artificial cavities and do so even when the landscape itself is highly developed, implying that they are primarily cavity-limited (Stanback et al 2011). While the effects of competition with eastern bluebirds were dramatic, we were interested in the importance of competition with similarly sized competitors, which overlap more completely in cavity size and degradation requirements in old growth pine forests (Blanc and Walters 2008). Although eastern bluebirds occur in old growth pine forests (Blanc and Walters 2008), Carolina chickadees (*Poecile carolinensis*), a size-matched passerine with a similar ecology to that of brown-headed nuthatches, are also prevalent (Blanc and Walters 2008).

Nesting success of brown-headed nuthatches (both probability of a successful nest and number of young fledged) increased with group size (Chapter 2) and experiments revealed that the

increase in nesting success with group size was stronger on high than low competition territories. This demonstrates that competition is an important selective agent on group size and that living in larger social groups can rescue brown-headed nuthatches from the detrimental impacts of competition (Chapter 2).

This paper focuses on how group living enables brown-headed nuthatches to defend their nest cavities form same-sized competitors. First, we used models to examine the behavioral components of group defense against heterospecific competitors, conspecific competitors, and potential predators, documenting group defense by different classes of individuals and asking how defensive behaviors scale with group size. By presenting heterospecific models of both predators and competitors, we were able to demonstrate that aggression to both types of threats was cooperative and intense compared to responses to a non-competitor model. Using observational data of naturally occurring competitive interactions, we determined the relationship between group size and time to detection of a heterospecific competitor as well as time to eviction once a competitor was detected. These data allowed us to examine how the behaviors, their effectiveness, and individual investment in defense (per capita mobbing effort) are influenced by group size.

Methods

Study site and population monitoring

We conducted this study on six golf courses located near Davidson, Mecklenburg Co., North Carolina, USA (35° 30’ N, 80° 50’ W), during the breeding season (February-Mid June) of 2014.

Golf courses consist of stands of shortleaf pine (*Pinus echinata*) or pines mixed with common southeastern deciduous trees (*Cercis canadensis, Acer rubrum, Fagus grandifolia, Quercus alba, Quercus falcate, Liriodendron tulipifera, Liquidambar styraciflua, and Cornus florida*) separated by open fairway spaces. Understory is non-existent due to active management and a thick layer of pine straw. The open understory mimics southeastern pine forests immediately post-burn, which is optimal for brown-headed nuthatches (Cox and Slater 2007). All nest boxes on the study site were equipped with small (26 mm) holes that excluded eastern bluebirds (*Sialia sialis*), and other larger competitors from nest building. This provided opportunities for brown-headed nuthatches to coexist with Carolina chickadees, forming a competitive community that is similar in composition to that found in native, pine habitat. In natural habitats, Carolina chickadees and other small birds would be able to access the cavities created or chosen by brown-headed nuthatches as nest sites, although eastern bluebirds and other large competitors would not. All nests were in Schwegler® "woodcrete" nestboxes with an interior diameter of 12 cm, mounted 1.75 m high on aluminum poles with ERVA® stovepipe-style predator guards. Boxes were separated by an average of 300 m, approximately the width of a brown-headed nuthatch territory (Cox and Slater 2007).

With the aid of 12 field assistants we monitored 138 groups of brown-headed nuthatches on all six sites for four years as part of a larger study. In addition to having a color-banded population, monitoring for 4 years prior to 2014 allowed us to know the ages of many individuals as well as the social pedigrees of group members. Adults were captured in mist nets, or through ambush on their nests, and nestlings were removed from nests for not more than 30 min (age 12-16 days). Once we had the birds in hand, we took morphological measurements, a small blood sample (≤

25 ul), and banded them with a unique set of three color bands and a metal leg band from the USFWS. After banding and measuring, adults were released onto their territory and nestlings were returned to their nests. Blood samples were used to genetically sex individuals using PCR amplification with the sexing primers 2550F and 2718R, followed by gel electophoresis (Fridolfsoon and Ellegren 1999).

Once a week we censused all brown-headed nuthatch territories for marked individuals to assess group size and group composition. All censuses were conducted between 0600-1000 h EST and involved searching territories, locating groups visually or aurally, and reading color bands to acquire individual identities. To facilitate location and encourage individuals to descend low enough for easy identification, we frequently used a small handheld speaker to play a male brown-headed nuthatch territorial call through an apple iPod mini. The territorial call we played was a five-minute loop of a call from the Macaulay Library, recorded in Florida, and thus unfamiliar to all individuals censused. Calls were never played for more than five minutes and brown-headed nuthatch groups showed no habituation to playback. Once we found the group and identified individuals present, we recorded those we saw as present and those we did not see, but had seen in the past, as absent.

Experimental examination of group defense

In 2014, we subjected all nesting brown-headed nuthatch groups (size 2-6) to three behavioral trials (Fig. 1). The experimental treatments were a black rat snake model (predator), a male brown-headed nuthatch model (conspecific competitor), and a Carolina chickadee model (heterospecific competitor). The black rat snake model was a rubber snake; the Carolina

chickadee and brown-headed nuthatch models were oven-dried birds that had been found dead in nest boxes. Models were placed on the nest box when no brown-headed nuthatch group member was in view. The snake was placed directly on the top of the nest box; the birds were attached to a twig that was placed just above the top of the nest box. Each group was exposed to all three treatments sequentially, in a balanced order, with no breaks in between treatments, when their nestlings were a week old. We chose the nestling stage to minimize the potential for extra-pair fertilization risk, which could otherwise influence male brown-headed nuthatches' responses to male conspecifics.

Figure 1: Experimental setup. Examples of the brown-headed nuthatch, Carolina chickadee, and snake model setup.

Trials lasted five minutes from when we had the first brown-headed nuthatch in view. During the trial we also played a territorial call of either a brown-headed nuthatch (nuthatch trial), or a Carolina chickadee (chickadee trial), through a small portable speaker and iPod mini. Calls played were five-minute loops of a call recorded in Florida by the Macaulay library, and thus

unfamiliar to all individuals tested. During the snake trial we also placed the speaker near the box and turned it on to control for the effect of minimal speaker static and the presence of a novel object (the speaker) near the nest. We randomly selected the model we began our first trial with, and then cycled through model types in a specified order to create a balanced design. We recorded audio and video for all trials, from a distance of 10 m using a Samsung HMX-590 video camera. In a separate audio recording we recorded running commentary on the locations and actions of all birds detected on the territory outside of the camera range and their actions. Later, using the audio and video recordings, we scored the number of birds detected in the territory during the trial, the time each bird spent mobbing the model, and the total time (of a possible five minutes) any bird mobbed the simulated invader. For the purpose of scoring videos, we defined mobbing as giving a conspicuous mobbing call and/or flying back and forth over the nest box within 2 m of the presented model.

Behavioral watches at nests to assess effectiveness of group defense

In 2013-2014 we observed 18 nests per year, six of each group size (2-4), during nest building, incubation, and the nestling stage to study the frequency of intrusion by heterospecific competitors and the form and outcomes of group defense. Once the first census was complete in February, groups were selected out of candidate groups of the appropriate size using a random number generator. Each selected group was watched once a week until it either successfully fledged young or the group ceased nesting for the season. Watches lasted for one hour, and occurred throughout the day, although most (64%) were after 1200 h EST. Nests were observed from a distance of 20 m and we recorded all nest visits by any species, writing down species identity, the purpose of the visit, and, if the individual was a brown-headed nuthatch, its band

combination. As this study was conducted on golf courses where people pass by all the time, brown-headed nuthatches were used to people and resumed feeding young a mean of 3.56 ± 0.52 min (N =115, ranging 0 to 29 min) after we set up to watch, despite the lack of a blind. We also recorded latency to detection of an intruder by at least one member of the group, aggressive interactions in the vicinity of the nest, latency to eviction or departure of the intruder, and the amount of time individual brown-headed nuthatches spent chasing intruders away from their nest. We defined detection of an intruder as the time a group member first gave a mobbing call or approached the intruder within 2 m.

Statistical analysis

All statistical analyses were performed in R version 2.13.1. For analysis of the experimental data, we performed generalized linear mixed models (GLMMs) using the package lme4 with group ID treated as a random factor to determine how group size effected total mobbing effort (sum of times all individuals in the group spent mobbing the model) and total mobbing time (time in the possible five min. trial any group member was mobbing the model). We used a Poisson error distribution for these models as our explanatory variable was constrained in its distribution (group sizes ranged from 2-6) and the response variables displayed a standard Poisson distributional pattern with many zero, or near zero events. We also used an ANOVA with a post-hoc Tukey HSD test, to determine if brown-headed nuthatch groups allocated their mobbing efforts differently based on model type.

For the behavioral watches at nests (no models presented, and group sizes ranging 2-4), response variables included number of visits to the nest box per hour by competitor species with, and

without, brown-headed nuthatch retaliation, and the length of time it took the group to chase the intruder off their territory once they responded to it. Because of the preponderance of zeros present in the distributions of all response variables, we used a Poisson distribution to model these variables. Because all groups in the nest watch analysis were watched repeatedly, group identity was treated as a random factor as was site identity (golf course).

We used GLMMs with Poisson error distributions to analyze our experimental data on the number of individuals responding, the apparent breeding status of responsive individuals, the age of responsive individuals, and the proportion of the group that responded to model presentations, because the distributions of all response variables skewed towards zero. GLMMs allowed us to determine if larger groups generally had more individuals present at the time of the trial presentations. We calculated the proportion of the group (as defined in weekly censuses) that was present at the time of the trial, and angularly transformed these proportions for parametric analysis of whether larger groups had a lower proportion of their group present during defensive trials against competitors and predators. We distinguished breeding males, breeding females, male helpers, male co-breeders, and female co-breeders using the following operational definitions: 1) Breeding males had bred in that location in the past, were genetically sexed as male, spent the most time accompanying breeding females, were most responsive to census playback, and were behaviorally dominant to all other group members; 2) Breeding females were behaviorally subordinate to other group members, were seen incubating, and were the only genetic female present in the group. 3) Co-breeding males were immigrants into focal group, sexed as genetically male, or stayed in a natal group when their mother was replaced by an immigrant female. 4). Females were considered co-breeding when more than one female was

present in the focal group regardless of relatedness to focal males. Extra pair paternity is common enough that the absence of unrelated males within group would present little barrier to reproduction. 5) All group members genetically sexed as male and not fitting these definitions of breeding or co-breeding were considered helpers. Cox and Slater (2007) found only one group with an incubating female helper, and suggest that almost all groups contain a single breeding female and a breeding male assisted by male helpers. The genetic data available supports this familial structure (Haas et al. 2009, Han et al. 2015). We then compared mobbing effort across classes of individuals. Finally, we compared time spent mobbing by the breeding male, breeding female, co-breeding males, co-breeding females, and helper males across all group sizes, treating group ID as a random factor, and including individual age as a covariate. These analyses also used a Poisson error distribution.

Results

Description of behaviors in natural competitive interactions

Brown-headed nuthatches upon sighting a competitor (house wren or Carolina chickadee) near their nest cavity immediately flew towards the intruder. If the competitor fled the brown-headed nuthatch(es) pursued it, often following very closely behind the invader. These chases would extend for several minutes as the competitor flew around the group's territory and only ended when the competitor completely left the area the brown-headed nuthatch group typically defended. If the competitor fled too slowly the brown-headed nuthatches physically attacked it, pecking the intruder and grappling with it such that both individuals fell to the ground. During these fights group members dive-bombed the grappling combatants, called loudly, and occasionally pecked the intruder as well. Eventually the competitor escaped, and was then pursued off the territory as described above.

Species involved in naturally occurring competitive interactions

Brown-headed nuthatches were extremely defensive of their nest cavities during the breeding season and attacked all comers, even non-competitors. Aggressive interactions occurred in 28% of nest watches (N= 387 hours) with a mean of 0.66±1.30 natural competitive interactions per watch hour. The majority (88% of 254) of aggressive interactions occurred with heterospecific competitors, including eastern bluebirds (52%), Carolina chickadees (28%), house wrens (4%), house sparrows (*Passer domesticus*, 2%), and tufted titmice (*Baeolophus bicolor*, <1%). Only a small percentage (2%) occurred with brown-headed nuthatches. A number of predators known to prey on nestlings or adults or parasitize brown-headed nuthatches were also aggressively repelled (4% of aggressive interactions), including a Cooper's hawk (*Accipiter cooperii*, <1%), red-bellied woodpeckers (*Melanerpes carolinus*, <1%), downy woodpeckers (*Picoides pubescens*, 1%), brown-headed cowbirds (*Molothrus ater*, 1%), and blue jays (*Cyanocitta cristata*, <1%). The remainder of aggressive interactions (8%) were directed at harmless species that perched on the top of a brown-headed nuthatch nest. Species chased included a northern cardinal (*Cardinalis cardinalis*), northern mockingbirds (*Mimus polyglottos*), a Carolina wren (*Thryothorus ludovicianus*), a yellow-bellied warbler (*Abroscopus superciliaris*), a chipping sparrow (*Spizella passerine*), American robins (*Turdus migratorius*), and an eastern phoebe (*Sayornis phoebe*).

Measures of defense based on model presentation and playback

Brown-headed nuthatch groups spent no longer mobbing competitor models than they did predator models (Time any group member mobbed the model during the five minute trial: Tukey HSD, df=204, p=0.94), and spent significantly less time mobbing the brown-headed nuthatch

model than the snake or Carolina chickadee model (ANOVA, $F = 4.83$, $df = 204$, $p = 0.008$). Brown-headed nuthatch group mobbing intensity was not greater for competitor than predator models (Sum of mobbing time by all group members: Tukey HSD, $df=204$, $p=0.94$), but they mobbed the conspecific model much less intensely (ANOVA, $F = 5.55$, $df = 204$, $p = 0.004$).

Group size and intensity of defense

The total time at least one brown-headed nuthatch was mobbing increased with group size both for predator and competitor models (1.45 ± 0.16, $z = 8.78$, $N = 129$, $p \leq 0.001$, Fig. 2a), indicating that the model was defended against for a longer period of time in larger groups. Total mobbing time increased with group size when groups mobbed a conspecific as well, despite the decrease in mobbing intensity directed toward the conspecific model (0.70 ± 0.03, $z = 24.23$, $N = 75$, $p \leq 0.0001$). The sum of time spent mobbing by all group members (total mobbing effort) was significantly positively affected by group size (1.56 ± 0.18, $z = 8.55$, $N = 129$, $p \leq 0.001$) when they were mobbing competitor and predator models (Fig. 2b). Conspecifics were also mobbed for longer periods when they approached nests of larger groups (Total mobbing time: 1.12 ± 0.03, $z = 44.33$, $N = 75$, $p \leq 0.001$). Considering only the birds we detected on the territory at the time of the trial as the immediate group size, the z scores for both mobbing effort (1.11 ± 0.02, $z = 52.73$, $df =370$, $p \leq 0.001$) and mobbing time (0.88 ± 0.03, $z = 31.53$, $df =370$, $p \leq 0.001$) increased with immediate group size; use of immediate group size thus strengthened the association between group size and defensive capacity of brown-headed nuthatch groups.

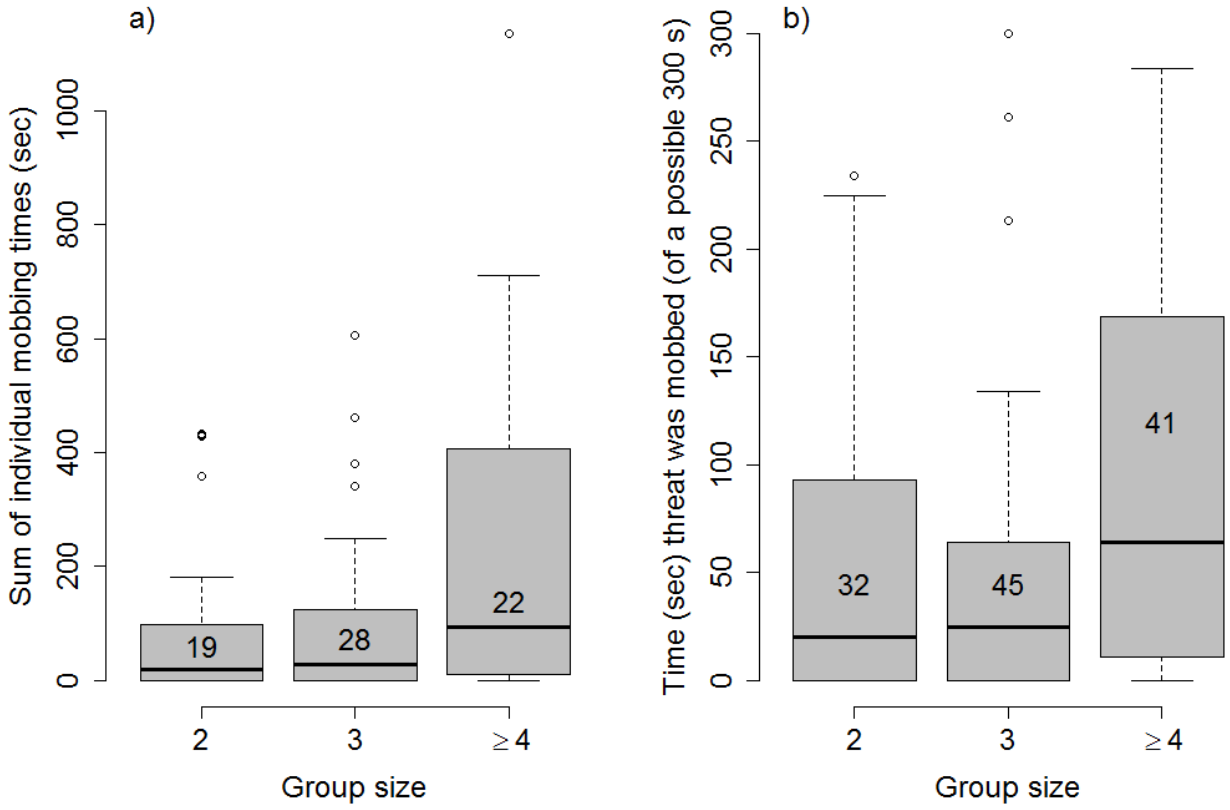

Figure 2. Total mobbing effort and time model was mobbed for group sizes of 2-4+ birds. Includes only predator and competitor trials. Sample sizes are written inside the boxes for each group size. Group size refers to the maximum number of brown-headed nuthatches detected on the group's territory during weekly census visits. Because of small sample sizes groups of five and six were lumped into category, group size ≥4. a) Sum of all group members' mobbing time includes times they overlapped with other individuals. b) Time any Brown-headed nuthatch spent mobbing the model, excluding times of overlap with other birds.

Effectiveness of group defense

During nest watches, group size had a negative effect on the probability that a group would have an intruder investigate their nest site without their knowledge and retaliation (-0.28±0.13, z = -2.10, df = 240, p = 0.03). Furthermore, in natural competitive encounters it took larger groups less time than smaller groups to chase intruders from their territories, such that they spent less time in the potentially dangerous act of fighting with heterospecifics, and intruders had less time

to inflict damage on the nest (-0.22±0.10, z = -2.27, df = 240, p=0.02, Fig. 3). The decline in latency to expulsion of intruders occurred at a group size of four birds.

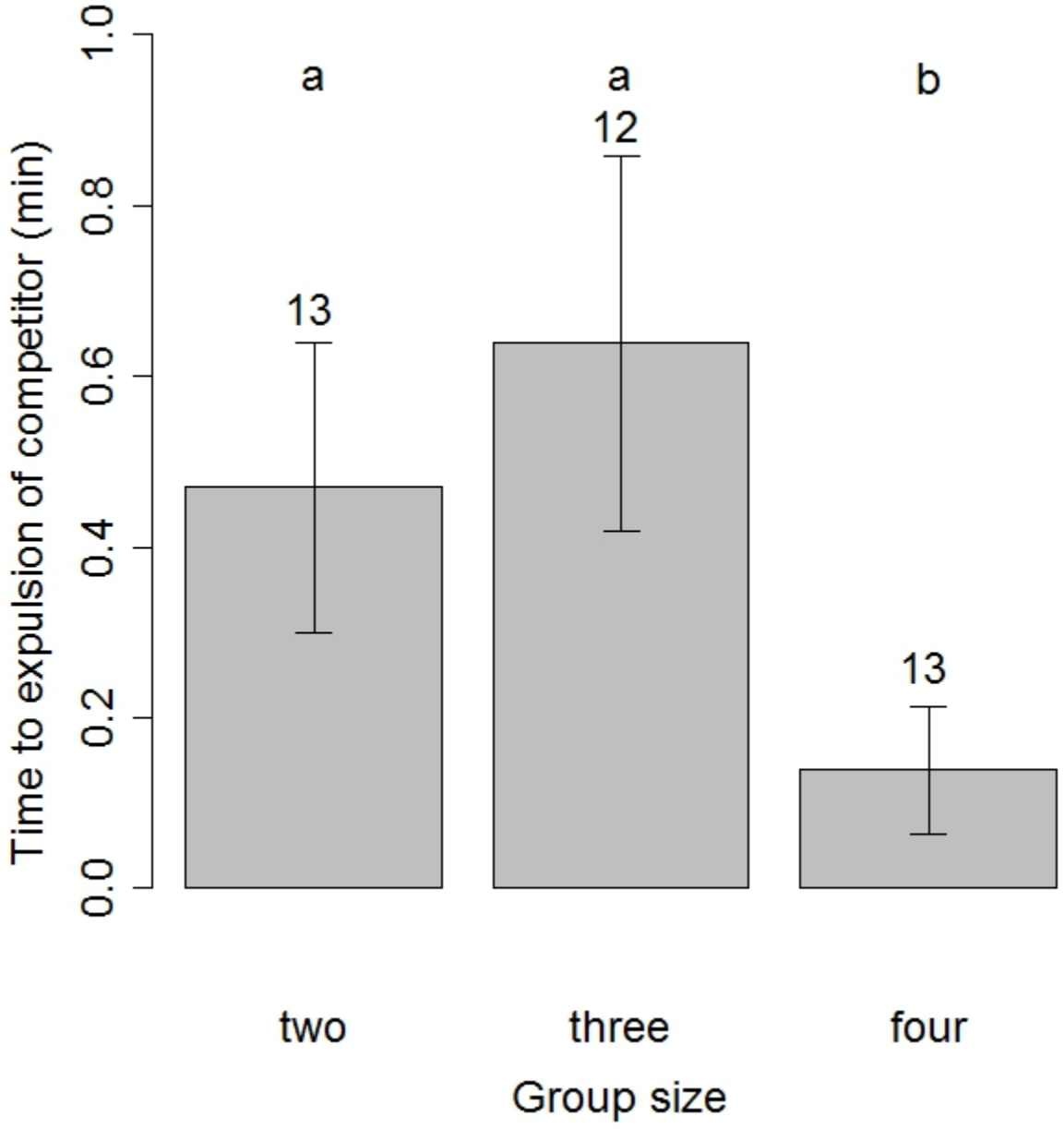

Figure 3: Time between interspecific competitor arrival and expulsion by brown-headed nuthatch group size. Bars that do not share a letter are statistically different at α=0.05 level. N= 202 watch hours. Error bars (±1 SD). Time (in minutes) it took the brown-headed nuthatch group to chase a competitor away from their nest site and off their territory.

Costs to Individuals

Even though not all group members were present for all competitor and predator trials, we consistently detected more individuals on the group territory at the time of the trials for larger than smaller groups (0.19 ± 0.05, $z = 3.63$, $df = 370$, $p \leq 0.001$, Fig. 4a).

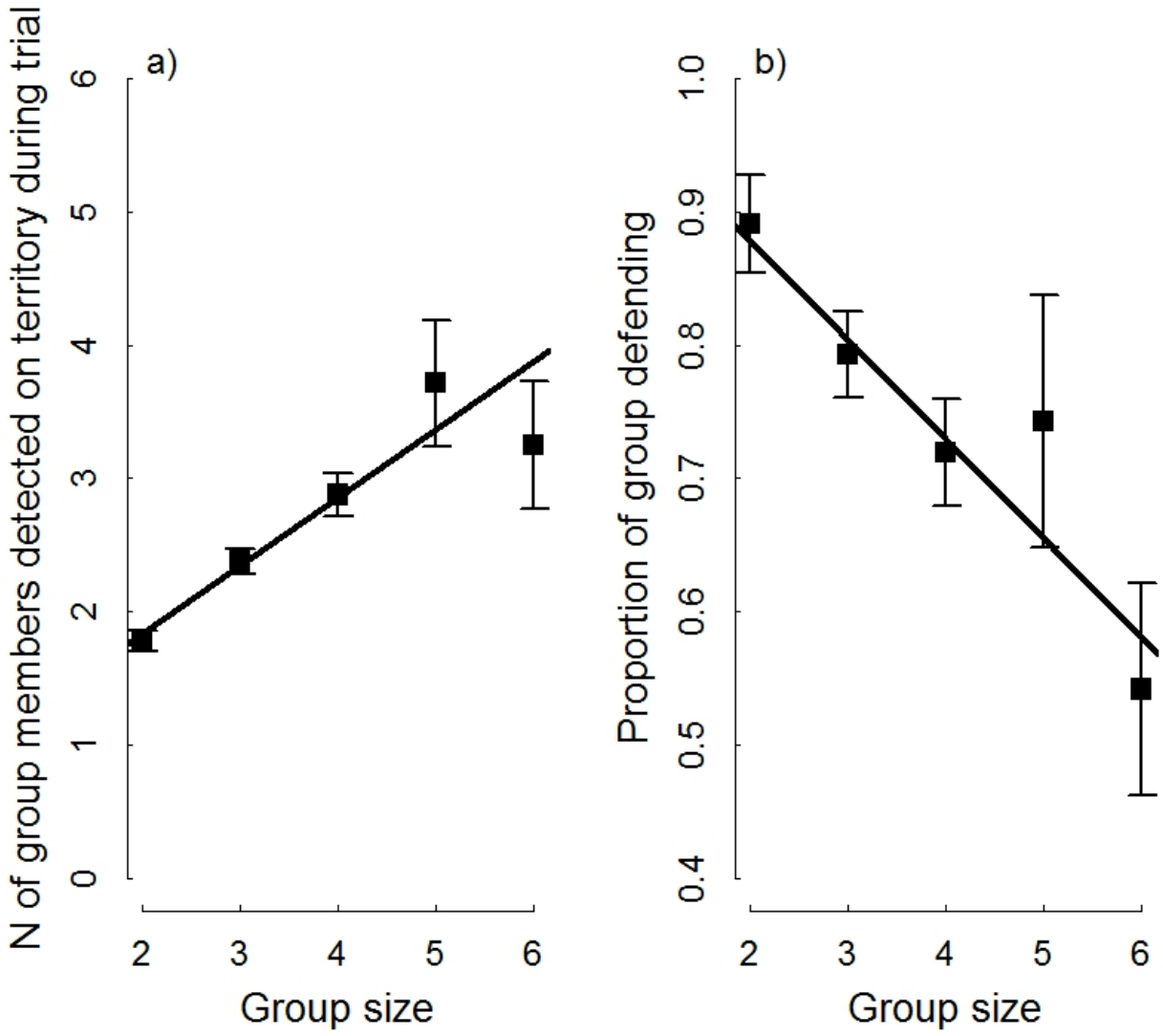

Figure 4: Number of individuals participating in defensive trials for brown-headed nuthatch groups of varying sizes. Includes only predator and competitor trials. Plotted values are the means ± SE. Group size refers to the maximum number of brown-headed nuthatches detected on the group's territory during weekly census visits. a) Compares brown-headed nuthatch group size to the number of brown-headed nuthatches detected (visually or aurally) during the trial presentations. b) Gives the proportion of maximum group size (detected during censuses) engaged in defense of the nest in competitor and predator trials.

Despite consistently presenting a larger defensive force, larger groups had a significantly lower proportion of their members participating in defensive trials against heterospecific competitors and predators (-0.17±0.04, $t = -3.94$, $df = 370$, $p \leq 0.001$, Fig. 4b). Breeding males participated in all mobbing events, however, breeding females were absent 12% of the time and one or more helpers were absent 45% of the time.

Age had a significant effect on mobbing efforts (GLMM, N = 258, 0.11 ± 0.01, $z = 12.9$, $p \leq 0.0001$), with older individuals spending more time mobbing than younger individuals. Group size had no effect on mobbing effort (GLMM, N = 258, 0.34 ± 0.27, $z = 1.3$, $p = 0.21$), but there was a significant interaction between group size and individual status (GLMM, N = 258, 0.02 ± 0.01, $z = 2.4$, $p = 0.02$). Helper males (GLMM, N = 62, 3.64 ± 1.23, $z = 2.96$, $p \leq 0.01$), but not other classes (Breeding males: GLMM, N = 75, 0.56 ± 0.41, $z = 1.37$, $p = 0.17$; Breeding females; GLMM, N = 75, 0.92 ± 0.57, $z = 1.59$, $p = 0.11$; Co-breeding males: GLMM, N = 42, -0.13 ± 0.92, $z = -0.15$, $p = 0.88$), increased their mobbing efforts as group size increased. Females spent significantly less time mobbing than males (GLMM, N = 258, -0.48 ± 0.04, $z = -12.44$, $p \leq 0.0001$). Even when using sex and age as covariates, individual status had a significant effect on mobbing effort (GLMM, N = 258, -0.35 ± 0.03, $z = -13.1$, $p \leq 0.0001$). Breeder males mobbed most, then co-breeding males, then helper males (Fig. 5). Individual status also impacted female effort, with female breeders mobbing more than female co-breeders (Fig. 5). All individuals responded consistently to each threat type, there was no interaction between type of threat (competitor/predator) and mobbing response based on status (no specialization, GLMM, N = 258, -0.02 ± 0.01, $z = -1.89$, $p = 0.06$). Although status did have a significant effect on individual age (GLMM, N = 258, -0.12 ± 0.02, $z = -5.19$, $p \leq 0.0001$), with

females and helper males tending to be younger than breeding and co-breeding males, there was no significant difference in the average age of groups of different sizes (GLMM, N = 258, -0.03 ± 0.03, z = -1.12, p = 0.26).

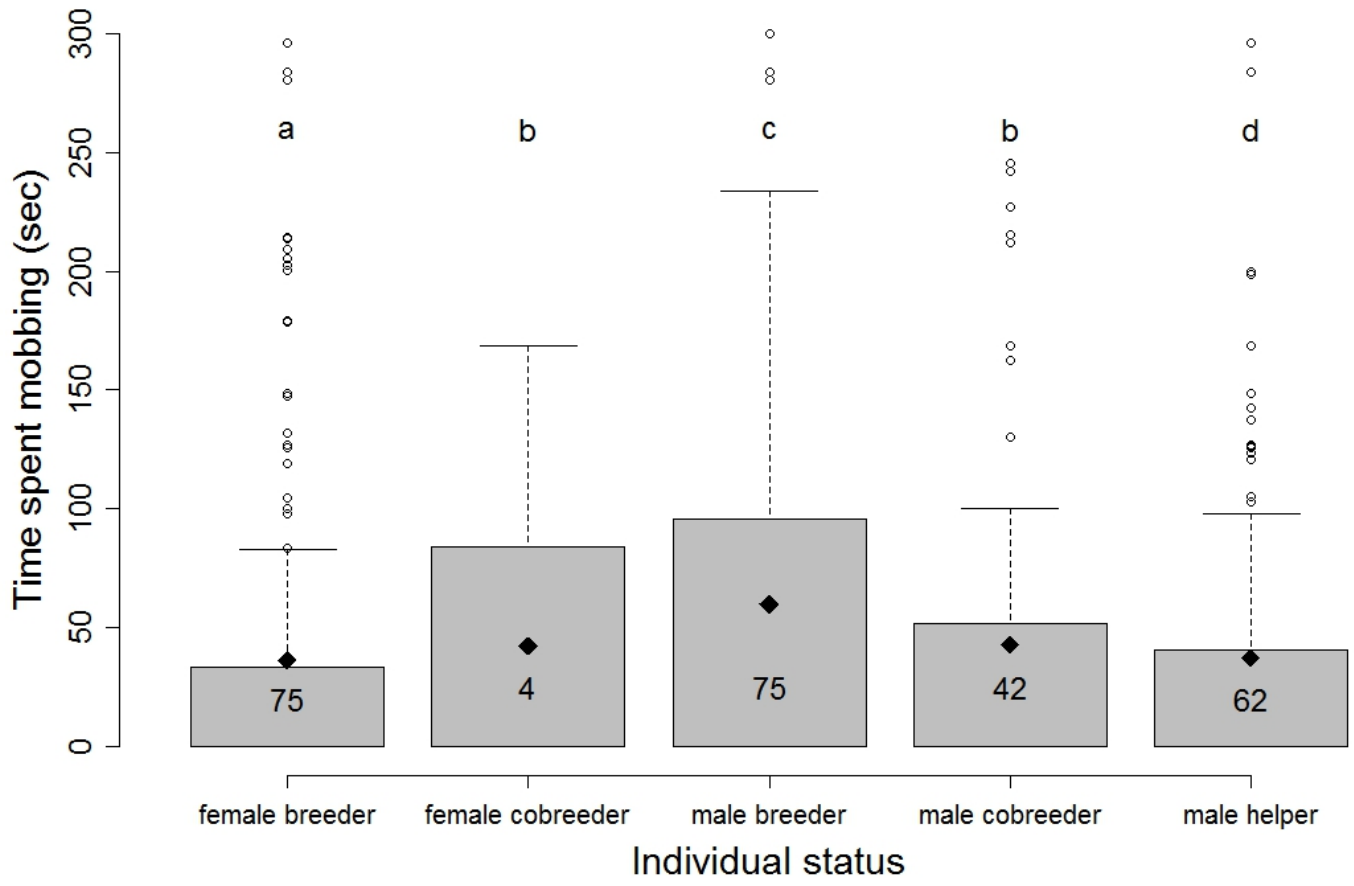

Figure 5: Time spent mobbing by different classes of brown-headed nuthatches. Boxes that do not share a letter are statistically different at the $\alpha = 0.05$ level. Includes only predator and competitor trials. Sample sizes (N = individuals of that status and sex) are written inside the plot. Individual status refers to the sex and group position of a nuthatch individual. Black diamonds indicate means for each status.

Discussion

Our results demonstrate how group defense against heterospecific competitors and predators manifests in a cooperatively breeding bird. The combined effort that brown-headed nuthatch groups invested in defending against heterospecific competitors and predators was greater than for conspecific competitors. This suggests that heterospecific competitors may be a greater threat than are conspecifics, and that they are similar to predators in their level of threat. The similarity in combined effort mobbing predator models and heterospecific competitor models provides further evidence that brown-headed nuthatches perceive heterospecifics as a serious threat. Behaviorally, predators and competitors elicited the same responses in brown-headed nuthatches (loud mobbing calls and extended mobbing of the model by all individuals present, including repeated dive bombing flights and the occasional direct attack). This find is similar to findings for cichlids and social shrimp (Toth and Duffy 2005, Taborsky and Limberger 1981), in which cooperative defense against heterospecific competitors is similar in intensity to defense against predators.

The increased intensity of mobbing (sum of times all individuals in the group spent mobbing the model) with group size, provides support for the idea that groups of more than a pair have increased defensive effort, even when these measures only included the subset of group members that were detected on the territory during the trial. Larger brown-headed nuthatch groups not only exhibited increased mobbing effort, they were more successful at defending their nests from naturally occurring competitors, expelling intruders more quickly than smaller groups (Fig. 3). Together with prior findings of an effect of group size on nesting success (Chapter 2), these results suggest that the behaviors associated with group defense have been under selection, not

only by predation but also by heterospecific competition. Particularly compelling in this regard is our finding that time to eviction of intruders declines with group size.

Collective defense against heterospecific competitors is not unique to this system. Eusocial snapping shrimp collectively snap at heterospecific intruders and are more likely to retain a sponge host than less social species (Toth and Duffy 2005, MacDonald et al. 2006). In tropical wrens (*Campylorhynchus spp.),* meerkats (*Suricata suricatta*), and southern red bishops (*Euplectes orix*), having extra mobbers beyond a pair increases the success of defense against predators (Rabenold 1990, Graw and Manser 2007, Brown and Lawes 2007). In a comparative phylogenetic analysis, Feeney et al. (2013) discovered that transitions to cooperative breeding are associated with brood parasitism, suggesting that defense against brood parasites might promote the evolution of cooperative breeding, although this idea has yet to earn broad acceptance in the field. In combination with these other studies, we have shown that collective defense plays a larger role in the evolution of cooperative breeding in vertebrates than we have hitherto considered (Chapter 2), and that it should, where important, be incorporated into the cost term when testing Hamilton's rule (1964).

The group size effect on latency to eviction provides evidence for the idea that auxiliaries play a critical role beyond simply feeding young; we suggest that they may be vital to the ability of breeders within the group to carry off a successful nest. Groups responded collectively and cooperatively to nest threats, with larger groups spending significantly longer times mobbing in response to threat, both in terms of the sum of all group members' mobbing times (a measure of the intensity of counter-attack) and in terms of the total amount of time the predator/competitor

experienced mobbing by one or more birds (a measure of the duration of the counter-attack) (Fig 2a, b). The association between group size and mobbing effort was stronger when only birds detected on the territory during experimental trials were considered. It is not clear whether undetected birds were sitting quietly somewhere in their territory, in which case they benefitted by reducing their efforts in a larger group, or whether undetected birds were foraying off territory and were unable to participate in group mobbing. As any bird visibly foraging on the territory at the time of trial was included in the mobbing group, the possibility that birds not present were foraying seems more likely. That the breeding male was never absent and the breeding female was rarely absent during a trial (12% of trials), whereas co-breeders and helpers were frequently absent (45% of trials), reinforces the theory that most absent individuals were out seeking a breeding vacancy. Regardless of which benefit non-participants were gaining from their absence, it is clear that individuals in larger groups pay a lower per capita defensive cost than individuals in smaller groups, not because the defenders work less hard, but because groups mount a robust defense with some members not participating at all. Such load-lightening as group sizes increase is one of the most commonly reported effects of cooperative breeding, although it is generally considered only in terms of provisioning efforts, not defenses or future direct fitness benefits (Hatchwell 1999).

Variation in defensive behavior with sex and breeding status within the group

Breeding status and sex within brown-headed nuthatch groups appears directly related to investment in mobbing of heterospecific competitors and predators (Fig. 5). Breeding females spent the least amount of time mobbing, breeding males the most, and helpers and co-breeders fell in between, with co-breeders offering more mobbing effort than helpers (Fig. 5). Recently,

much research attention has focused on how and why individuals within cooperative groups behave differently in different contexts (Stiver et al. 2006, Mares et al. 2012, Ozqul et al. 2014). Generally, researchers refer to different fitness optima to explain why some individuals offer more aide than others (Stiver et al. 2006, Mares et al. 2012, Ozqul et al. 2014). We speculate that females contribute least to mobbing because of their high investment in brooding and incubation (Cox and Slater 2007), and because females, as the rarer sex (Cox and Slater 2007, unpublished data) have more opportunities to gain direct fitness, and thus more to lose when contributing to dangerous activities like mobbing predators and competitors (Childress and Lung 2003, Ferretti et al. 2014). Conversely, as males vastly outnumber females, they have a lower chance of successfully reproducing in the future, making the current breeding effort more valuable to them and motivating their increased effort. It is also possible that males have more energy to invest in mobbing, as they neither incubate, nor brood the young (Withgott and Smith 1998). The decrease in investment as within group status declines from breeder, to co-breeder, to helper is consistent with inclusive fitness theory, as the fitness gains available to each type of individual also decline. Breeders produce more offspring in the nest than co-breeders, although both types of individuals regularly contribute genetically to the current brood (Chapter 4). Breeders, on average, are defending more of their own offspring than co-breeders, and thus have more at stake if the brood is lost. Helpers, however, rarely sire offspring within the group and thus generally have only indirect fitness gains at stake (Chapter 4). As indirect fitness returns are lower than direct fitness returns (Chapter 4), helpers are less motivated to risk their safety by mobbing dangerous predators and competitors.

Finally, helpers spent more time mobbing per individual as group sizes increased, indicating, at the very least, that brown-headed nuthatch effort and thus cost per individual of mobbing, does not decrease for helpers as group sizes increase. This increased investment with group size may be due to decreased risks when more individuals responded to the threat, through predator/competitor confusion or threat dilution. (Garay 2009, Olson et al. 2013, Ioannou et al. 2009). Threat may be reduced due to a mini-selfish-herd effect (Garay 2009, Olson et al. 2013, Ioannou et al. 2009) or because larger group size decreases the likelihood of attack, as it does for predators of the chestnut crowned babbler (Sorato et al. 2012). Only helping males are sensitive to risk dilution in this manner, however, likely because their fitness returns from mobbing are smaller, and thus small differences in the perception of risk can alter the fitness equation. Helper males are also the youngest individuals (modal age: yearling) and thus ought to place the highest value on future direct fitness gains, which, again, would make them more sensitive to risk.

We have already shown that heterospecific competition negatively effects brown-headed nuthatch probability of nesting and fledging success (Chapter 2). Having more group members, however, ameliorates the costs of heterospecific competition to brown-headed nuthatches by increasing their probability of nesting and their fledging success when they face heterospecific competition (Chapter 2). The increase in fledging success is great enough to increase per capita female fledging success with group size (Chapter 2). Here we demonstrate the mechanism by which larger group sizes rescue brown-headed nuthatches from the detrimental effects of heterospecific competition. Primarily, that the behavioral responses to heterospecific competitors are similar to the behavioral responses to predators. Brown-headed nuthatches increase their mobbing effort expended and mobbing effectiveness with group size. Thus larger groups detect

and evict heterospecific intruders more rapidly. A common ecological constraint (nest cavities) appears to work in combination with heterospecific competition to reduce the costs of joining a group by reducing the direct fitness benefits of breeding independently (low success rate, Chapter 2). These reduced costs elevate the relative importance of indirect fitness benefits (sensu Brown 1980) of helping to raise close kin and, possibly, the future direct and indirect fitness benefits arising from increased survival of both helpers and their recipient kin. According to Cox and Slater (2007) helpers are almost always close relatives of the breeding male, most commonly sons or brothers, making them highly likely to be genetically related to the nestlings by at least 0.25 and, if their mother is still present, as high as 0.5. These relatedness values make indirect fitness gains to helpers from defending the brood likely, but as in most cooperative breeders, these indirect fitness benefits will rarely compensate for failing to breed independently. Additional research is needed to estimate relatedness values among helpers and the young they help to feed and defend and to examine current and future direct and indirect fitness benefits to helpers (sensu Brown 1980, Meade et al. 2010).

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CHAPTER 4

Extra-group breeding, within-group helping, joint nesting, and polygynandry in Brown-headed nuthatches

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ABSTRACT

Brown-headed nuthatches (*Sitta pusilla*) have previously only been studied in the southern portion of their range. Here, we report on the group compositions, relatedness structures, and direct reproduction within and outside the group for a nest box nesting population at the northern edge of the species range. Brown-headed nuthatches in our population exhibit all possible combinations of cooperative social behavior, including helping, joint-nesting, cobreeding by both sexes, and even polygynandry. Layered on top of this complex within-group structure, we found high levels of both extra-pair paternity, and intraspecific brood parasitism. Despite this complex structure, a single breeding pair still monopolized a majority of the reproduction both within, and outside of their home social group. Relationships to group mates mediated reproductive success for both cobreeders and helpers; direct fitness of cobreeders increased when same sex relatives were present in the group, whereas helper direct fitness decreased when relatives were present. The direct fitness of female breeders also increased when male relatives were present in the social group.

Keywords: Cooperative breeding, polygynandry, cobreeding, intraspecific brood parasitism, extra-pair paternity

INTRODUCTION

Cooperative breeding, when three or more individuals work together to raise young, is a relatively uncommon breeding system that occurs in approximately 9% of bird species and 3% of mammal species (Cockburn 2006, Soloman and French 2007). Cooperative groups vary from the simple pairs with one or more nonbreeding helpers (Woolfenden and Fitzpatrick 1984) to polygynandrous groups like Acorn woodpecker groups (*Melanerpes formicivorus*), which can

consist of up to three joint-nesting females, up to four cobreeding males, multiple non-breeding helpers of both sexes (Haydock and Koenig 2002, Haydock and Koenig 2003), and plural breeders, in which multiple pairs exist within a group and helpers move among a selection of pairs to help at the nest (Brown 1970, Riehl 2011). Researchers have recorded many benefits to breeding in cooperative groups for auxiliaries in these groups, including increased survival probability (Sorato et al 2012, Meade and Hatchwell 2010), increased indirect fitness through the production of non-descendent kin (Conner et al. 2004, Blackmore and Heinsohn 2007, Klauke et al. 2013, Emlen and Wrege 1991, Lloyd et al. 2009, Rowley et al. 1991, Meade et al. 2010), increased success in extra- pair offspring production (Young et al. 1987), and territory inheritance (Woolfenden and Fitzpatrick 1984). These benefits may be, and often are, however, considerably lower than the fitness payoffs of breeding independently. Thus, cooperative systems tend to occur where external constraints limit the ability of individuals to breed independently, such that helpers are making the best of a bad job (Dickinson and Hatchwell 2004).

Understanding the cooperative breeding system of a particular species of population requires first characterizing the social and genetic composition of the groups. Extra-pair paternity, where it occurs, can greatly shift the interpretation of a cooperative system. A premier example is that of the Superb fairy-wren (*Malurus cyaneus*) in which groups that were formerly thought to be incestuous, were shown to be non-incestuous based on 72% offspring having been sired by extra-pair males (Double and Cockburn 2000, Dunn and Cockburn 1999). Apparent breeders within groups were actually non-breeding helpers. At the same time, where extra-pair fertilizations occur, direct fitness benefits can arise for helpers to the extent that they reproduce either outside or within their social group. As a result, studies of the fitness benefits of helping

must necessarily begin with an assessment of the genetic mating system based on inference of parentage using molecular tools. Understanding how social and genetic parentage influence individual reproductive opportunities both within the focal group, and outside of it, is a critical first step to uncovering the inclusive fitness components (direct and indirect) that must be accounted for to understand the ultimate causes of social group formation and cooperative breeding.

Brown-headed nuthatches are small, cavity-breeding passerines native to the American southeast, where they specialize on old growth pine forests maintained by frequent fires, preferring highly degraded snags for nesting sites (Withgott and Smith 1998). Brown-headed nuthatches are cooperative breeders, showing not only the typical pattern of female-biased dispersal seen in passerines (Greenwood and Harvey 1982), but also living on year-round territories where they exhibit extreme natal philopatry of sons and group sizes ranging from 2 to 6 birds (Cox and Slater 2007). Although their social group structure has been characterized (Cox and Slater 2007), and a genetic paternity study has recently been published (Han et al. 2015), both studies examined a population near the southern extent of the species range, and reported relatively small group sizes. Little is known about the social and genetic structure of Brown-headed Nuthatches in northern end of their range and no prior study has examined social behavior and paternity in nest box populations.

There are two reasons why it is important to investigate the social and genetic structure of Brown-headed Nuthatches in nest box populations in the northern part of their range. First, in northern parts of their range, current conservation strategies depend heavily on the use of nest boxes that exclude Eastern bluebirds (*Sialia sialis*) (Stanback et al. 2011). Use of nest boxes, which often involves using predator guards, can alter the demography of pairs leading to more

surviving offspring and longer adult survival, which can increase the potential for cooperative breeding. Despite Brown-headed Nuthatches being listed as a species of least concern on the IUCN red list, their populations have been in decline since the 1970s (Robbins et al. 1989). Moreover, according to recent climate models released by scientists at the Audubon society, Brown-headed nuthatches are climate-endangered and likely to lose at least 50%, and likely 95% of their breeding range to climate change by 2080 (Langham et al. 2015). Audubon has responded to the threat to this species by implementing wide ranging efforts in North Carolina to place Brown-headed nuthatch sized nest boxes throughout the state (http://nc.audubon.org/conservation/make-little-room-brown-headed-nuthatch).

Nest boxes have been successful in enabling Brown-headed Nuthatches to re-colonize disturbed habitats, such as golf-courses, where most of the long-leaf pines have been removed (Stanback et al. 2011). Second, we expect changes in cooperative breeding with changes in latitude because clutch size tends to be larger in the north (Jetz et al. 2008). This can also lead to larger group sizes and demographic effects on the sizes and compositions of groups (more helpers). Brown-headed nuthatches are known to lay smaller clutches in Florida than in more northern areas of their range (McNair 1984). Both the use of nest boxes and latitudinal patterns suggest that the social system of Brown-headed Nuthatches will differ between the northern and southern parts of their range.

In general, understanding the social behavior of a species, and the factors that influence group living, is enhanced by studying multiple populations in different regions; for example only by studying Acorn woodpeckers simultaneously in Arizona, New Mexico and California, was the full extent of their social behavior, and the intertwined importance of habitat characteristics, revealed (Koenig and Stacey 1990, Chapter 14, Ed. Koenig and Stacey). Finally, as few groups

were genotyped in the Florida population, this study allows us to assess relatedness within groups and identify sources of auxiliary reproduction with a resolution not previously possible. In this study, we characterize the genetic mating system and its potential impact on direct and indirect fitness for helpers in Brown-headed Nuthatches and assess the basic structure and relatedness of groups in a nest box population in North Carolina, where cooperative groups appear more common than in the southern part of their range. We also estimate the importance of two potential avenues to direct fitness for auxiliaries in cooperative social groups: 1) direct fitness through extra-pair mating outside the social breeding group and 2) direct fitness achieved by mating within the social breeding group (cobreeding).

METHODS

General Methods

We conducted this study on six suburban golf courses located near Davidson, Mecklenburg Co., North Carolina, USA (35° 30’ N, 80° 50’ W), during five breeding seasons (February-Mid June 2011-2015). Golf courses were separated by a mean of 12.1±1.3 km. The fairways of our study sites were surrounded by residential houses in an open matrix of trees dominated by shortleaf pine (*Pinus echinata*) or pines mixed with common southeastern deciduous trees (*Cercis canadensis, Acer rubrum, Fagus grandifolia, Quercus alba, Quercus falcate, Liriodendron tulipifera, Liquidambar styraciflua, and Cornus florida*). Understory is typically non-existent due to suburban lawns and actively managed pine straw beds. The open understory mimics southeastern pine forests immediately post-burn which is optimal for Brown-headed nuthatches (Cox and Slater 2007). Although the golf course habitat is suitable for Brown-headed nuthatches, it differs from the spaces they occupy in Florida in several ways, primarily through tree cover

being patchier (interrupted by fairway spaces), and the absence of any Cyprus swamp regions or proscribed burning. All nest boxes on the study site were equipped with small (2.6 cm) holes to promote Brown-headed nuthatch nest building and exclude larger species. We used Schwegler "woodcrete" nestboxes with an interior diameter of 12 cm, mounted 1.7 m high on aluminum poles and equipped with ERVA stovepipe-style predator guards. Nest boxes were checked weekly, and no nest was removed until the conclusion of the breeding season. Boxes were placed on golf courses between 2011 and 2015 for a total of 292 nest boxes (per year) on average 300±15 m apart (range 254:1035). Inter-box distance thus approximated 300 m, the diameter of a Brown-headed nuthatch territory (Cox and Slater 2007).

We monitored all groups of Brown-headed nuthatches on these six sites for five years as part of a larger study. Adults were captured in mist nets (or through ambush on their nests) and nestlings were briefly removed from nests (age 12-16 days). Adults were captured either in early in the breeding season prior to the start of egg laying, or during the second week of nestling presence to avoid prompting nest abandonment. Once we had the birds in hand, we took morphological measurements (relaxed wing cord, tarsus length, and mass), a small blood sample ($\leq$ 25 ul), and banded them with a unique set of three color bands and a metal leg band from the USFWS. All blood samples were collected by the authors. After banding, we released adults back onto their territory and returned nestlings to their nests. Nestling banding records allowed us to determine ages of individuals. All nestlings subsequently observed in the population were of a known age based on their birth year. Individuals banded as adults were given an age of two in their first year of capture, as this is when most known age breeders aquired their breeding vacancy.

Determination of Group Size, Social Status, and Social Relatedness

Brown-headed nuthatch groups were censused twice a year to determine group composition and group size, once at the beginning of the breeding season, and once at its conclusion. All censuses were conducted between 0600-1000 am. Individuals were identified through the unique combination of colored leg bands. To census groups, we searched their territories; either locating the group visually or aurally and then using binoculars (Nikon 8 X 42) to acquire individual identities. To facilitate location and encourage individuals to descend low enough for easy identification, we frequently used a small handheld speaker to play a male Brown-headed nuthatch territorial call through an apple iPod mini. The territorial call we played was a five min loop of a call recorded in Florida and retrieved from the Macaulay library, and thus unfamiliar to all individuals censused. Censuses also allowed us to determine social status of all group individuals.

Social status was inferred first by banding records and behavioral data. Social status based on banding data was deemed to be important because it captures the information that the birds actually have about each other; in general, evidence suggests that recognition of relatives is based on prior experience in cooperatively breeding birds (Hatchwell et al. 2001, Riehl and Stern 2015). If a male or a female had previously bred in a group it retained its socially-inferred status as a breeder. If the male/female did not share within group breeding opportunities with others of the same sex this individual was called the solo breeder. In the absence of this information, the oldest male, which was both the primary individual that responded to simulated territorial intrusions by coming closest to the speaker, and also mate-guarded the apparent breeder female, was assigned solo breeding male status. Immigrant males were assigned either solo breeder or cobreeder status. They were considered the solo breeder when all other males in the group were socially related to, and thus would be committing incest with, the breeding female and they were

considered a cobreeder when they joined a group with a male that had bred in the territory in previous years such that neither was a socially-inferred relative of the breeding female. Males in the group that were socially related to the breeding female were categorized as helpers, and were generally younger than, and born during the tenure of, the social solo breeding male or male cobreeders. These social characterizations follow the recommendations of the most recent synthesis on cooperative breeding in vertebrates (Koenig et al. 2015, Chapter 20, Ed. Koenig and Dickinson). Similarly, a female was assumed to be the solo breeder if she was the primary incubator of the clutch; solo breeder females tended to be the oldest female present in the group. Females that migrated into a group from another territory were also designated as solo breeders if they were the sole female present or cobreeders if another breeding female was already established in the group, because they could mate non-incestuously with the breeder male. The prevalence of extra pair paternity (EPP) in this species (Han et al. 2015) suggests that existing in a group without non-incestuous options should not limit within group reproductive opportunities for female auxiliaries, thus all females remaining in the group during the breeding season were considered cobreeders. The designation cobreeder was used for either sex whenever multiple individuals within a group could breed non-incestuously. No attempt was made to distinguish status among potential cobreeders. The term solo breeder was only used for individuals that socially monopolized group reproduction.

Social relatedness was determined based on familiarity. Nestlings were considered socially related to all adults present in the group that raised them. Current breeders or cobreeders in the group were given a social relatedness value of 0.5 to the nestlings. Current helpers were given relatedness values of 0.5 to the nestlings when the helper had been raised by both breeders, and 0.25 when they were the offspring of just one breeder. Nestlings were also considered

socially related (at the 0.5 level) to all siblings within their natal brood. We compared socially inferred status and relatedness of individuals with those based on genetic assignments of parentage using only data from 2013 and 2014 when genetic information could be paired with social designations. We verified the behavioral designations with genetic data: 98% of males and 98% of females designated as main breeders produced a majority of the nestlings from their group (i.e. the male sired a majority of the nestlings and the female laid the majority of the eggs). The remaining 2%, although they did produce nestlings in the brood, did not sire the majority of the nestlings. We also confirmed that cobreeders participated in the reproductive attempt, with 22% (N = 124) of potential male cobreeders (those unrelated to the breeding females based on apparent genealogy) siring one or more nestlings in the brood and 63% (N = 18) of potential female cobreeders laying eggs that hatched in the nest. Only 9% of 190 male helpers sired nestlings within their group, and all such pairings were genetically, as well as socially, incestuous. Our confidence in these incestuous assignments is high, as for all but three cases (N = 16) the father of the incestuous breeding helper was also genetically sampled and excluded as the genetic sire.

Determination of Genetic Relatedness and Paternity/Maternity

In the summer of 2015, DNA was extracted from 1535 Brown-headed nuthatch blood samples collected over the previous four years (2011-2014) using the Qiagen DNeasy Blood and tissue kit. We optimized multi-plexing of PCR reactions using fluorescent dye-labeled primers based on amplification of 12 published microsatellite sequences (Haas et al. 2009). Optimization resulted in two PCR reactions with differing reaction temperatures and primer concentrations and similar run times. After PCR amplification we sent the resulting PCR products to Cornell's biotechnology facility for genotyping through phosphorescently labeled fragment analysis with

each well also containing the ladder LIZ to standardize sizes. The number of alleles we observed at each locus was similar, though not identical, to those recorded for the same species in Florida by Haas et al. (2009), with a mean of 26.25 alleles per locus. This indicated that even when full siblings were present in the set of candidate parents (as they undoubtedly were) we had a 2.1 X 10^{-12} chance of attributing the offspring to the wrong genetic parent. We examined allele frequencies using only breeding adults (N = 225), and found that frequencies deviated from Hardy–Weinberg equilibrium at half of the loci, but this was likely due to the structure of our dataset, which contained six subpopulations. Only two alleles, EF474468 and EF474472 showed strong evidence of the presence of null allele $n \geq 0.30$, and were dropped from subsequent analysis, all others had a low null allele probability $n \leq 0.05$. We manually scored alleles using the program Generous 9.0.

We used program Cervus 3.0 (Kalinowski et al. 2007) to infer parentage of the offspring, with the male potential parent pool including all sampled males in the population (on the specific golf course and alive) in the year the nestling in question was born. As golf courses were separated by an average of 12 km, and no individual was witnessed prospecting on a non-natal course before returning to the natal course (although between breeding seasons dispersal between golf courses did occur) and males were observed visiting territories up to 1500 m from their home territory, this selection of males represents a reasonable set of possible fathers. Cervus uses Monte Carlo simulations to assess confidence values for each assigned parent. For these simulations we specified that the population contained related males (full siblings or offspring) as potential sires in proportion to their prevalence in social pedigrees from our field data (2-9%). We also used field data to specify the proportion of individuals sampled (60-75%) and the number of candidate parents. We accepted the putative male as the genetic sire for 611 of 790

genotyped offspring (77%) using the following rules that were adapted from Webster et al. (2003) to compensate for the presence of many close relatives among potential sires, 1) The putative father must have two or fewer loci mismatched to the nestling's genotype. 2) The nestling and the putative father must have at least 6 loci genotyped to compare. 3) When two or more males met the first two criteria and were attributed at least 80% confidence of paternity we assigned the nestling to the male with the most complete genetic information and fewest mismatching loci. 4) If the third criteria did not result in a single definitive sire we examined the social information of both males. Preference was given to potential sires within the social group that produced the nestling over males not in the group, or to neighboring males over males from more distant territories. 5) If rule three and four failed to establish a sole genetic sire we split assigned paternity between the two potential sires (each received credit for 0.5 nestlings).

Although this is perhaps not the most biologically correct method to distinguish paternity, it nonetheless has been suggested to compensate for the presence of many close relatives in the sample of potential parents (Jones and Ardren 2003). As this only occurred in 9 of the 611 assignments it should have, at worst, a minor impact on downstream results. Nestlings whose parentage could not be determined with these rules were dropped from subsequent analyses. As females are uncommon as helpers and disperse farther (Cox and Slater 2007) they are less likely to suffer the spatially clustered genetic structuring that made determining paternity so fraught (Haas et al. 2009). Maternity was therefore attributed to the female present in the group unless another spatially close female was assigned maternity with 95% confidence by CERVUS.

To calculate genetic relatedness between individuals we used an equation derived by Queller and Goodnight (1989) and adapted to calculate relatedness between individuals by Oliehoek et al. (2006). The equation counts shared alleles to calculate likely relatedness values,

assuming a background level of shared alleles in a population. The R package "demerelate" uses this equation to generate relatedness values (r_{xy}) between all individuals in a user specified group. Calculating relatedness values this way allowed us to determine relatedness between individuals even when paternity assignment was inconclusive.

Analysis of Within-group and Extra-group Maternity and Paternity

All statistical analyses were performed in R version 3.2.1. Means are presented ± standard error. Unless otherwise stated in the results section data for analyses were drawn from years 2013-2014. We analyzed whether the number of young sired, or laid, within a group was impacted by the relatedness structure of the group using generalized linear mixed models and the R package "lme4". The within group offspring model included group identity nested within year as a random factor and, because of the preponderance of individuals contributing zero offspring, used a Poisson error distribution. Males and females were analyzed separately. Beginning with a full model containing all factors we thought might be important to the generation of within-group young (group size, social status, relatedness to group members, and individual age), as well as all possible interactions, we created a model set that ranged in complexity from the full model, down to a null model which contained only the nested random effects (group identity within year). All possible combinations of factors were included in model set. We then performed AICc model selection (using the R package "AICcmodavg") to determine which model best described the variation in the data set and report the significances and effect sizes from the factors included in the most supported model. Where more than one model received substantial support (Δ AICc within 2) model averaging was conducted to produce the final model using the R package "MuMIn".

The factors contributing to extra-group maternity or paternity were also analyzed with a set of generalized linear mixed models and AICc model averaging. Males and females were analyzed separately, and a Poisson error distribution was used for all models. Again, all factors of interest included group size, social status, relatedness to group members, and individual age. We began with a full model which included all factors and all possible interactions, and ended with a null model which contained only the nested random effects (group identity within year). All possible combinations of factors were included in model set. We then performed AICc model selection (using the R package "AICcmodavg") to determine which model best described the variation in the data set and report the significances and effect sizes from the factors included in the most supported model. Where more than one model received substantial support (Δ AICc within 2) model averaging was conducted to produce the final model using the R package "MuMIn".

RESULTS

Group Size

The mean group size was 3.1 ± 1.0 adults and ranged from 1-6 individuals (n = 556 group-years censused 2011- 2015, Figure 1). The majority (59%) of groups had more than a pair of adults tending the nest. Number of groups varied from year to year, and there was a small, but statistically significant effect of number of groups on group size (GLMM, group ID within year as a random effect, n = 556, 0.02 ± 0.005, t = 4.07, $p \leq 001$), with larger groups being slightly more common in years with more groups present.

Group Compositions

Brown-headed nuthatch groups were socially complex (Figure 2). We used all groups studied in 2013 and 2014 (n = 276) to assess the frequencies of various group compositions based on

socially inferred relationships (Figure 2). When a male's social mother disappeared, helper males often remained in the natal group and ascended to cobreeding status. When a helper's social father disappeared dispersal was much more common. Ascension to cobreeding status was statistically more likely to occur when the opposite sex social parent was replaced than when the same sex parent was replaced (GLMM, 1.01 ± 0.22, $z = 4.52$, $n = 677$, $p \leq 0.001$, Figure 3A). Female cobreeders were much rarer than male cobreeders, despite all female auxiliaries being considered cobreeders by virtue of their ability to mate non-incestuously outside the natal group at all times while laying eggs within the social group (Figure 3B). As group size increased, the prevalence of cobreeding also increased (Fisher's exact test, $n = 206$, $p \leq 0.05$). Groups could contain both cobreeders and helpers, and 13% of groups did so, with all groups of six and the majority of groups of five containing both (62%, $n = 24$).

Within Group Parentage

The mean percentage of offspring sired within group by socially designated solo breeder males (irrespective of group size) was $54 \pm 3\%$ and the mean percentage within group nestlings attributed to solo breeder female was $71 \pm 5\%$. Socially designated cobreeding females produced $27 \pm 6\%$ of within group nestlings, while cobreeding males were responsible for only $17 \pm 2\%$ of within group offspring. Males categorized socially as helpers had the lowest success rate, siring only $5 \pm 1\%$ of within group offspring. All breeding helpers were socially, as well as genetically, incestuous. For both males (GLMM, $n = 315$, -1.92 ± 0.25, $z = -7.76$, $p \leq 0.001$) and females (GLMM, $n = 48$, -1.93 ± 0.40, $z = -4.76$, $p \leq 0.001$), socially designated solo breeders produced a significantly greater proportion of within-group young than did cobreeders. Additionally, males categorized socially as helpers sired significantly fewer offspring within the

group than either solo breeding males (GLMM, n = 315, -3.41 ± 0.25, z = -13.66, $p \leq 0.001$), or cobreeding males (GLMM, n = 315, -1.48 ± 0.26, z = -5.66, $p \leq 0.001$).

Intraspecific Brood Parasitism and Extra-pair Paternity

Both intraspecific brood parasitism and extra-pair paternity occurred in Brown-headed nuthatch groups. Nestlings frequently had extra pair sires (55%, n = 475 nestlings); similarly over half of all broods contained at least one nestling from an extra-pair sire (54 %, n = 211 broods). Intraspecific brood parasitism was less common; only 22% of nestlings were from eggs laid by intraspecific brood parasites. However, 38% of groups raised at least one brood parasitic offspring.

Solo breeding males engaged in extra-pair mating, with 28% of a total of 106 breeding males siring young in other groups' nests. Cobreeding males were similarly, but not more successful at siring extra-group young than solo breeding males (23% of 124 cobreeding males, Fisher exact, n = 230, p = 0.56). Helpers were also successful at siring extra-pair offspring: 18% of 190 helpers sired one or more nestlings outside their natal group; this percentage was not statistically lower than that for cobreeding males (males also born in the group, but for which breeding with the dominant breeding female would not be incestuous) (Fisher exact, n = 314, p = 0.40). Helper males were also not significantly less successful at siring extra-pair young than solo breeding males (Fisher exact, n = 296, p = 0.11). Similarly, many solo breeding females, 42% of 44, engaged in intra-specific brood parasitism (ISBP). Cobreeding females also laid eggs in other nests at a high rate with 26% of a total of 18 cobreeding females engaging in ISBP. Intraspecific brood parasitism was not statistically more common in solo breeding females than cobreeding females (Fisher exact, n = 64, p = 0.59).

Social and Genetic Relationships of Helpers and Cobreeders

Most male cobreeders (77% of 124 cobreeding males) were socially related (either brothers or sons) to their male breeding partner, giving them an average relatedness of 0.38 to their cobreeding partner (Figure 3A). Brother/brother pairs cobreeding together were nearly as common (45% of cobreeders breeding with social relatives) as father/son cobreeding pairs (55% of cobreeders breeding with social relatives). Despite the prevalence of EPP and egg dumping, the genetic story does not differ substantially from our findings based on socially inferred status; 64% of male cobreeders were genetically related to their male breeding partner (Fisher's exact test male social vs. male genetic, $n = 124$, $p = 0.43$). Examining genetic, rather than social, data only lowers the average relatedness between male cobreeders to 0.32 (Figure 3A). Just as when considering social relationships, brother/brother pairs cobreeding together were slightly less common (46% of cobreeders breeding with genetic relatives) than father/son cobreeding pairs (54% of cobreeders breeding with genetic relatives).

Among female cobreeders, 72% of 18 were socially related (either sisters or daughters) to their female breeding partners (Figure 3B). Sister/sister pairs cobreeding together were much less common (12% of females cobreeding with social relatives) than mother/daughter cobreeding pairs (88% of females cobreeding with social relatives). Because of the opportunities for extra-pair copulations, female cobreeders could be socially related to the main male breeder (daughters), and many were (61%). Although cobreeding females were more commonly related to the cobreeding female than to their male partner, a large portion were socially related to both breeding partners (50%), giving an overall social relatedness score (only male + only female + both breeders) between cobreeding females and their breeding partners of 0.39. Genetic relatedness between cobreeders, however, was lower, likely due to the prevalence of EPP and

egg dumping (Figure 3B). Only 33% of cobreeding females were genetically related to the main male breeder (Fisher exact test, male social vs. male genetic, $n = 18$, $p = 0.38$), and only 33% were genetically related to their cobreeding female partner (Fisher exact test, female social vs. female genetic, $n = 18$, $p = 0.26$). Genetic sister/sister pairs never co-bred together; all cobreeding partners that shared a genetic relationship were mother/daughter pairs. No females were genetically related to both breeding partners (Fisher exact test, both breeding partners social vs. both breeding partners genetic, $n = 18$, $p \leq 0.01$). This relatedness structure produced an average genetic relatedness of female cobreeders to their breeding partners of 0.34.

Based on socially inferred status, all helpers were male and assisted at the nest of at least one social parent. The vast majority of helpers (94%) assisted at the nest of both social parents, giving them an average social relatedness of 0.48 to the breeding adults (Figure 4), and helpers were significantly more likely to assist at nests where they were socially related to both parents, than at nests where they were socially related to only a single parent (Fisher exact, $n = 190$, $p \leq 0.001$). Because of the prevalence of EPP and egg dumping, however, the genetic story is substantially different. Helpers were significantly less likely to be genetically related to both parents than socially related to both parents (Fisher exact test, $n = 190$, $p \leq 0.001$). Only 32% of helpers were genetically related to both parents, lowering the average relatedness to the breeding pair to 0.28 (Figure 4). Moreover, helpers were significantly more likely to assist at a nest where they were genetically related to only a single parent than at a nest where they were genetically related to both parents (Fisher exact, $n = 190$, $p \leq 0.05$). This pattern was primarily driven by genetic relatedness to females (49% of 190 helpers were related to the female only), as helpers were significantly more likely to assist at the nest of their genetic mother, than at the nest of their genetic father (Fisher exact, $n = 190$, $p \leq 0.001$). Only 1% of the 190 helpers we studied were

genetically related to only the breeding male. Although all helpers were socially related to the breeders they assisted, genetically, a nontrivial minority were related to neither the breeding male, nor the breeding female (19% of 190 helpers) due to intraspecific brood parasitism and extra-pair paternity.

Effect of Social Status (Main Breeder, Cobreeder, or Helper) and Relationship to Breeders on Within Group Reproduction

We examined the effect of social status on within group reproduction. As group members were frequently closely related, breeding within the group carried a high risk of incestuous mating, particularly for auxiliary individuals. Therefore we also asked how incidences of within group reproduction were affected by social and genetic relationships. Since both individual age and group size can impact reproductive success, we used these factors as covariates in our generalized linear mixed models of the effect of social status and relationships to breeders on within group reproductive success. We ran models for each sex sepparately, using first social relationship values (i.e. based on social pedigrees) and then genetic allele sharing values to determine the relationships between group adults. For males, the most supported model based on social pedigrees indicated that social status had the largest impact on the number of within group young an individual male sired, followed by their relationships to group mates (GLMM, n = 420, 0.29 ± 0.37, z = 0.78, p = 0.43), group size (GLMM, n = 420, 0.15 ± 0.20, z = 0.76, p = 0.45), an interaction between social status and social relationships (GLMM, n = 420, -0.10 ± 0.15, z = 0.64, p = 0.52), an interaction between group size and social relationships (GLMM, n = 420, 0.11 ± 0.10, z = 1.10, p = 0.27), and finally individual age (GLMM, n = 420, 0.004 ± 0.09, z = 0.05, p = 0.96, Figure 5A). Only social status, however, had a significant effect on number of young sired within the group (GLMM, n = 420, -1.02±0.30, z = 3.35, $p \leq 0.001$), with main breeders

siring significantly more offspring within the group than cobreeders and helpers, and cobreeders siring more within group offspring than helpers (Figure 5A). When the model set was based on genetic allele sharing, rather than social pedigrees to establish relationships, results were similar, with social status again having the greatest impact on within group young sired, followed by an interaction between social status and genetic relationships, genetic relationships (GLMM, n = 420, -0.13 ± 0.29, z = 0.46, p = 0.65), group size (GLMM, n = 420, 0.22 ± 0.15, z = 1.47, p = 0.14), and individual age (GLMM, n = 420, 0.03 ± 0.09, z = 0.37, p = 0.71, Figure 5B, 5C). Both social status (GLMM, n = 420, -1.45±0.27, z = 5.27, $p \leq 0.001$, Figure 5B), and the interaction between social status and genetic relationships to group members predicted the number of within group young sired by males (GLMM, n = 420, 0.32±0.14, z = 2.31, $p \leq 0.05$, Figure 5C), with main breeders being most successful at siring within group young (Figure 5B). Genetic relationships to cobreeders or helpers had no effect on main breeder reproductive success, but for both cobreeder and helper males sharing more alleles with male group mates increased the number of within group young sired (Figure 5C).

For females, the best supported model based on social pedigrees indicated that social relationships had the largest impact on the number of within group young (Figure 6A), followed by social status (GLMM, n = 62, -1.70 ± 1.03, z = 1.65, p = 0.10, Figure 6B), group size (GLMM, n = 62, 0.19 ± 0.17, z = 1.10, p = 0.27) , an interaction between social status and social relationships (GLMM, n = 62, 0.33 ± 0.42, z = 0.77, p = 0.44), and finally individual age (GLMM, n = 62, 0.06 ± 0.12, z = 0.47, p = 0.63). Only social relationships, however, had a significant effect on number of young females produced within the group (GLMM, n = 62, -1.02±0.30, z = 3.35, $p \leq 0.001$, Figure 6A); females with male social relatives in the group had more within group young than females without male social relatives in the group. When the

model set was based on genetic allele sharing to infer relationships, rather than social pedigrees, results were similar, with genetic relationships having the greatest impact on within group young produced (GLMM, $n = 62$, 0.62 ± 1.06, $z = 0.58$, $p = 0.56$, Figure 6B), followed by an interaction between genetic relationships and group size (Figure 7C), female age (GLMM, $n = 62$, 0.11 ± 0.14, $z = 0.85$, $p = 0.40$), group size (GLMM, $n = 62$, -0.50 ± 0.40, $z = 1.25$, $p = 0.21$), social status (GLMM, $n = 62$, 0.42 ± 1.86, $z = 0.22$, $p = 0.82$), an interaction between social status and genetic relationships (GLMM, $n = 62$, -1.53 ± 2.46, $z = 0.61$, $p = 0.54$), an interaction between group size and social status (GLMM, $n = 62$, -1.16 ± 0.87, $z = 1.33$, $p = 0.18$), and finally a triple interaction between group size, genetic relationship, and social status (GLMM, $n = 62$, 1.25 ± 0.71, $z = 1.76$, $p = 0.08$, Figure 6C). Despite the pletora of factors relained in the best fit model, only the interaction between group size and genetic relationships to group members predicted the number of within group young produced by females (GLMM, $n = 62$, 0.46 ± 0.23, $z = 1.96$, $p \leq 0.05$, Figure 6C); females with relatives as helpers produced more young as group size increased, but females without genetic relatives as helpers produced fewer young as group size increased.

Effect of Social Status (Solo Breeder, Cobreeder, or Helper) and Relationship to Breeders on Extra-group Reproduction

Both males and females had opportunities to reproduce outside the group. We used model selection to determine how social status and relationships to breeders influenced male extra-group parentage. Models also included as covariates age and group size, as each could influence reproductive success. The most complex model contained the main effects social status and relationship to group mates based on social pedigrees, as well as the covariates age and group size, and all interactions thereof. AICc model selection produced a best supported model for males containing only the factors social status (GLMM, $n = 420$, -0.34 ± 0.13, $z = 2.57$, $p \leq$

0.01), age (GLMM, n = 420, 0.06 ± 0.09, z = 0.62, p = 0.53), and social relationships to group mates (GLMM, n = 420, -0.26 ± 0.10, z = 2.55, $p \leq 0.01$, Figure 7). Only male social status and relatedness to group mates had a statistically significant effect on the number of extra-group young males sired. Main breeder males sired more extra-group young than cobreeder males or helper males (Figure 7A). Males with social relationships to the main breeding female sired fewer extra-group offspring than males with no social relationships to the main breeding female (Figure 7B).

As with males the most complex model for females contained the factors social status and relationships to group mates, as well as the covariates age and group size, and all interactions thereof. We then used AICc model selection to determine the best supported model. The model that best explained female intraspecific brood parasitism contained the main variable of interest, social status (GLMM, n = 62, -0.51 ± 0.52, z = 0.97, p = 0.33), as well as female age (GLMM, n = 62, 0.21 ± 0.23, z = 0.90, p = 0.37) and group size (GLMM, n = 62, 0.18 ± 0.20, z = 0.89, p = 0.37). The variable social relationship to group members was not retained in the best supported model. However, the factors retained in best supported model were not statistically significant predictors of female interspecific brood parasitism.

DISCUSSION

Brown-headed nuthatches breeding in nest boxes in Davidson, NC exhibit a greater variety of breeding systems than found in any single population of cooperatively breeding birds. Their group sizes are larger than previously reported for populations in pine forests in Florida, and they showed every kind of kin-based cooperative breeding system studied to date: pairs with helpers, cobreeding by males and females within a group (and involving a single nest), and

polygynandry, where they have multiple breeding males and multiple breeding females at a single nest. Layered onto this system are a reasonable level of extra-group fertilizations (54% of nests) and an unusually high level of intraspecific brood parasitism for a small passerine bird (38% of nests).

What is most surprising about this system is that birds of both sexes and all statuses, including helpers, main breeders, and cobreeders, breed both within and outside their home group. Main breeding males had the greatest success at both within group reproduction and EPP, although it was also common for cobreeding males and helper males to sire extra pair young. Helper males were the least successful at within group reproduction, and their propensity to sire extra-group young was also most effected by their relationships with group members, primarily whether the breeding female was their mother. Helper males related to the breeding female sired fewer extra group young than helper males unrelated to her. For females, on the other hand, status had no effect on their probability of laying one or more eggs through intraspecific brood parasitism. Social status did, however, effect production of within group young, as main breeding females were responsible for a much larger proportion of the within group offspring than cobreeding females. All this complexity means that analyzing the inclusive fitness benefits for males and females is likely to be more fraught than previously recognized.

The proportion of groups larger than a simple pair was much greater in the Davidson, NC population (59%) than previously reported values on a populations in Florida (Cox and Slater 2007, range 10-32%). Increased group sizes however, were not accompanied by an increase in the density of groups in our population. We placed nest boxes on our golf courses in accordance with the distances between territories reported by Cox and Slater (2007), and this resulted in a nuthatch territory density on our golf courses of 0.17 territories/ha. This density is higher than

Cox and Slater (2007) reported for one of their populations (0.08 territories/ha), and lower than they reported for the other (0.33 territories/ha). The larger group sizes found in our study area could be the result of demographic effects, either through increased clutch sizes in the north, effects of nest boxes and predator guards on Brown-headed nuthatch fledging success, or our use of nest boxes with small entrances known to exclude Eastern Bluebirds and other large birds. Although clutch sizes were larger than found in Florida (5.04 ± 0.05, 575 nests, compared to 4.50 ± 0.80, 80 nests (McNair 1984)), fledging success was lower (3.62 ± 0.09, 575 nests, compared to 4.16± 1.45, 117 nests (Cox and Slater 2007)). It's possible that the lower fledging success found in our population is due to our greater ability to include early nest failures in the data set, which is more difficult in natural cavity settings. When fledging success was calculated using only nests that hatched one or more nestlings (those detected in a natural cavity study) our fledge success increased to (4.31 ± 0.07, 481 nests). Without similar measures for the Florida study system, we cannot rule out fledging success as a cause of larger group sizes found in our population.

The larger groups in our population were also more complex. We found evidence for the presence of cobreeders of both sexes. Some cobreeders were related to the other individuals in their groups, but a large minority (approx. 30%) were not (Figure 3). Unrelated auxiliaries have been reported before in Brown-headed nuthatches (Haas et al. 2010, Han et al. 2015), suggesting that cobreeding may be a common behavioral strategy in the species. Cobreeding was a common strategy for males in our population with over a quarter of all groups having two or more cobreeding males (Figure 2). Cobreeding females were much less common, occurring in only 4% of groups (Figure 2). As the sex ratio of adults in groups was highly biased towards males (unpublished data) this disparity may simply reflect the different breeding opportunities available

to males versus females. In many cooperative breeders with female biased dispersal, as occurs in Brown-headed nuthatches, female helpers (or cobreeders) are less common than males (Woolfenden and Fitzpatrick 1984, Haydock and Koenig, 2002; Haydock and Koenig 2003). In some, there appears to be a shortage of breeding females in the population, perhaps because females, in traveling longer distances, have higher mortality during dispersal and settlement (Dickinson et al. 2015). Females, if in short supply, will have more opportunities to form a breeding pair, and thus will have less incentive to enter into cobreeding partnerships that require them to the pay the costs of sharing reproduction. If the deficit of females is higher in our population than in Florida, we may see larger group sizes due to the combination of a shortage of nesting opportunities and a shortage of opportunities, particularly for males, to breed independently (Cox and Slater 2007, Han et al 2015).

Extra-pair Paternity

Both this study and a prior study of paternity in Brown-headed nuthatches showed high rates of EPP, suggesting that high EPP is a species characteristic (Han et al. 2015). Using the 99% confidence interval calculations suggested by Griffith et al. (2002) we found that the estimated levels of EPP likely overlap (54 ± 8.84 %, this study, 41 ± 16.49 %, Han et al. 2015). Thus Brown-headed nuthatches exhibit rates of EPP above the passerine mean of 20% (Griffith et al. 2002), and above the mean rate of EPP in cooperative breeders of 15% (Cornwallis et al. 2010). The only cooperative breeders with comparable rates of EPP are several species of fairy wrens, Western bluebirds (*Sialia mexicana*), Australian magpies (*Cracticus tibicen*), and Seychelles warblers (*Acrocephalus sechellensis*) (Cornwallis et al. 2010). These species present a bit of a puzzle because monogamy, and or low rates of EPP, have recently been linked to the evolutionary transitions to cooperative breeding in birds, insects (Hymenoptera), and mammals

(Hughes et al. 2008, Cornwallis et al. 2010, and Lukas and Clutton-Brock 2012). This evolutionary association between low rates of EPP (or monogamy) and cooperative breeding strengthens the benefits of helping in the system by increasing relatedness between helpers and the offspring they help. The hypothesis generally suggests that systems with high rates of polygyny, polyandry, polygynandry, and extra pair mating are derived systems evolved as a response to environmental conditions, in the case of insects, once cooperation had become fixed (Boomsma 2007, Cornwallis et al. 2010). EPP has only been genetically examined in one other species of nuthatch, the European nuthatch (*Sitta europa*), finding an EPP rate of 10% (Segelbacher et al. 2005). One other data point, combined with a disputed phylogeny (Pasquet 1998, Pasquet et al. 2014), and a lack of social structure information on Asian nuthatch species (Matthyson 1998), makes the relationship between paternity and transitions into or out of cooperative breeding impossible to resolve.

Male Reproductive Success

Individual males that managed to acquire and retain a breeding vacancy on their own produced more young, both within, and outside, their home social group than either cobreeding individuals, or helpers (Figures 5, 6, and 7). Extra group paternity may have favored males holding the main breeding position because females took cues from other females on male ability or genetic value (Double and Cockburn 2000, Dunn and Cockburn 1999). Higher quality males may also be more likely to acquire a breeding vacancy (Mönkkönen 1990, Lozano 1994, Pryke and Andersson 2003), thus females could be keying more directly on male quality (Otter et al 1998), which simply correlates with breeding position when choosing extra-group mates. Main breeders were also able to secure much of the reproductive potential of their group. This reproductive partitioning, however, may be primarily due to incest avoidance by either males,

females, or both sexes, rather than the result of a tug of war over reproductive skew (Magrath et al. 2004, Shen and Reeve 2010). When non-incestuous mating within the group was possible for males, many of them mated and successfully produced within-group young as cobreeders (Figures 5 and 6). In contrast, males related to the breeding female(s) rarely reproduced within the group. These patterns suggest that the primary governor of within group reproduction is an incest avoidance mechanism. Reproductive shares both within and without the group were predicted by social status with main breeders securing the most, then cobreeders, then helpers. Thus all individual may be reproducing directly in any given breeding season, suggesting that analysis of inclusive fitness benefits of helping, cobreeding, and singular breeding for males is far from straightforward.

Female Reproductive Success

Female Brown-headed nuthatches mirrored the patterns seen in males, with a few added complexities. As in males, solo female breeders were more successful than cobreeders at producing young within the group (Figure 6), however, unlike in males, solo female breeders and cobreeders were equally successful interspecific brood parasites. Group relationships were the strongest predictor of within group reproductive success (Figure 6). Breeding females had the greatest reproductive success when they had multiple male relatives as helpers (Figure 6). Cobreeding and having a female relative as a partner, however, decreased reproductive success. Overall this suggests that both inter-sex and intra-sex competition over reproductive shares occurred when females attempted to cobreed, reducing their share of reproduction as compared to solo female breeders.

Additionally, joint nesting females commonly experience a reproductive cost, often through resource loss via egg dumping (Riehl 2010, Koenig at al. 1995, Komdeur 1994), and Brown-headed nuthatches fit this general pattern, with cobreeding females experiencing roughly half the reproductive success of solo female breeders. However, we saw no evidence of egg dumping directly, although it is possible that it occurred in the early morning hours prior to our nest watches (E. Niemasik, personal observation). The large reproductive cost likely explains why female cobreeders were uncommon in the population, with only 4% of groups containing more than a single female. As the rarer sex females also had more options for direct reproduction, which also likely decreases the incentive to breed jointly (Cox and Slater 2007, Han et al. 2015, this study). Despite these costs and the general availability of breeding vacancies, however, each year a few females chose to nest jointly. It is possible that females used this method to queue for particularly high quality territories, or large groups. Group size had a large positive effect on female reproductive success, particularly in groups of relatives (Figure 6). In these circumstances inheriting the territory in a year or two may be worth the immediate fitness cost (Woolfenden and Fitzpatrick 1984). We tested this hypothesis post hoc by comparing group sizes for cobreeding males and females, but found no evidence that female cobreeders tended to occur in larger groups than male cobreeders (GLMM, normal error, -0.12 ± 0.31, $t = -0.37$, $p = 0.71$). Current group size, however, may not be the best estimate of territory quality and females may still be cobreeding only on the highest quality territories. More research is necessary to understand what prompts these rare females to joint nest.

Helper Reproductive Success

Helpers rarely reproduced within the social group and this lack appeared primarily to occur to avoid incestuously mating with their mother (Figure 4). Helpers were also least successful in

extra-group reproduction (Figure 7A), however, their relationship to the breeding female within their social group mediated this success (Figure 7B). Helpers socially descended from the breeding female were less successful in acquiring extra-pair mates than helpers with no social descent from the breeding female. This may be due to a choice by helpers to invest more in within group reproduction when closely related to the breeding female as breeding females with genetically related (more shared alleles) male helpers in their social group experienced a group size benefit, whereas females without male relatives in their social group experienced a cost (Figure 6C). Helpers likely face a trade-off between investing in indirect fitness returns within their group by feeding nestlings, or spending time investing in future direct fitness by searching for breeding opportunities (either extra-group copulations or breeding vacancies) away from the home territory. When males were closely related to the female the inclusive fitness returns are higher and likely lead to greater within group investment; when they were less related helper males were freed to be more "selfish" and appeared to invest less in group success (Figure 6C) and more in personal reproduction (Figure 7B). This pattern perfectly matches what kin selection and inclusive fitness theory would predict (Hamilton 1964, Russell and Hatchwell 2001). More research on provisioning effort as a function of relatedness to nestlings is needed to test this hypothesis for Brown-headed nuthatch helpers, but the data so far is quite suggestive.

Conclusions

Brown-headed nuthatch social groups in our population are complex, with cobreeding males common in the population, and more rarely, cobreeding females appearing as well. The division of reproduction within the group seems to primarily be determined by incest avoidance, although having male relatives in the group appears to increase within group reproductive success for both male and female breeders and the species is ripe for analysis with reproductive skew models.

Future work on the inclusive fitness of helpers and/or cobreeders needs to include their abilities to reproduce directly both within, and outside the focal social group. Both sexes of Brown-headed nuthatches have complex reproductive strategies including high levels of extra-group reproduction (extra-group mating for males and intraspecific brood parasitism for females). This complexity in genetic reproduction makes Brown-headed nuthatches a fascinating study organism for those interested in cooperation because it naturally creates variable relatedness structures within the population, which can then be experimentally mined for insights into the roles of relatedness, external constraints, and variable environments.

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FIGURES:

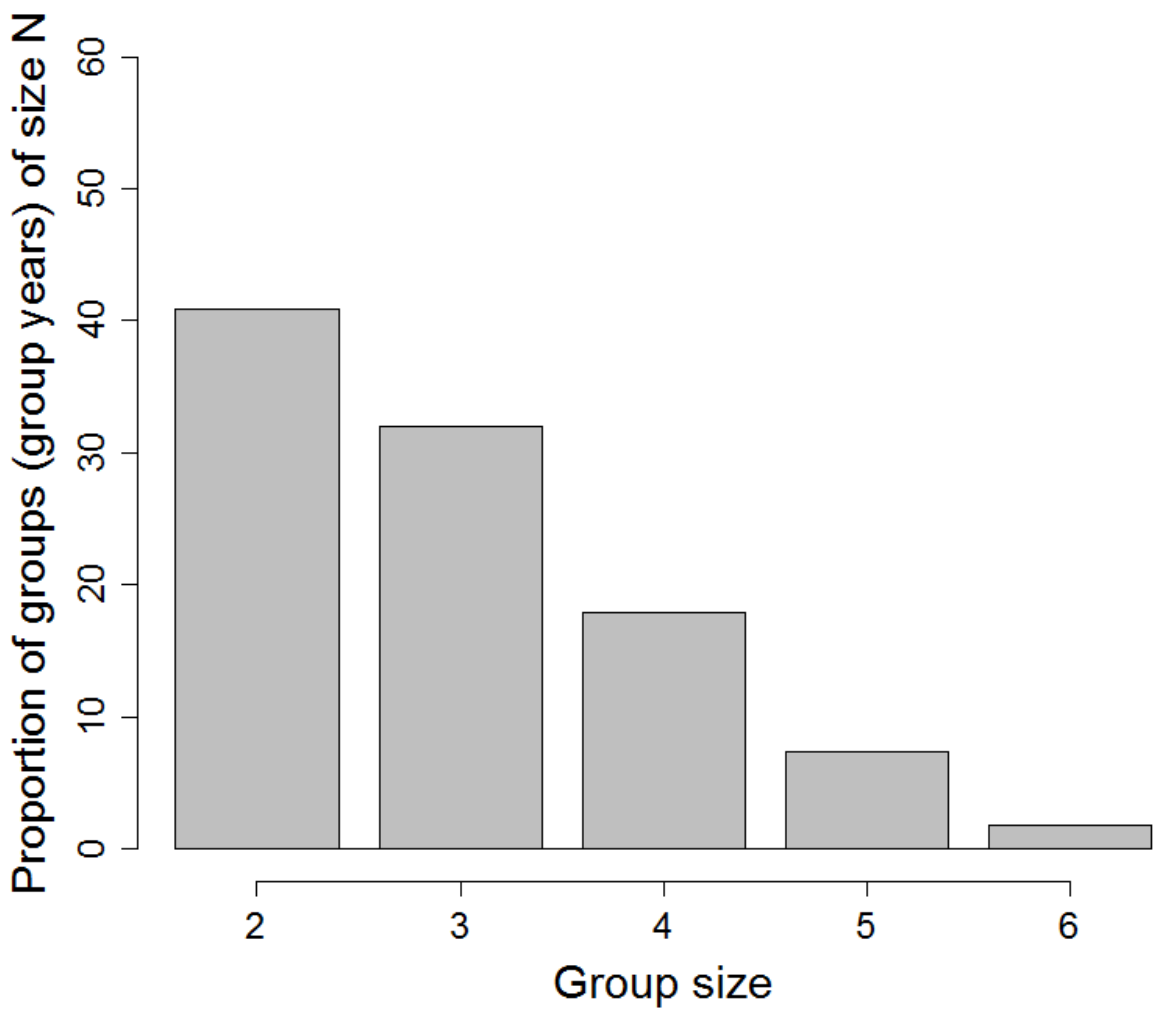

Figure 1. Distribution of brown-headed nuthatch group sizes. Shows proportion of groups of each size**,** based on census data collected from 2011- 2015. Group compositions changed between years so each group was included for each year (group-years).

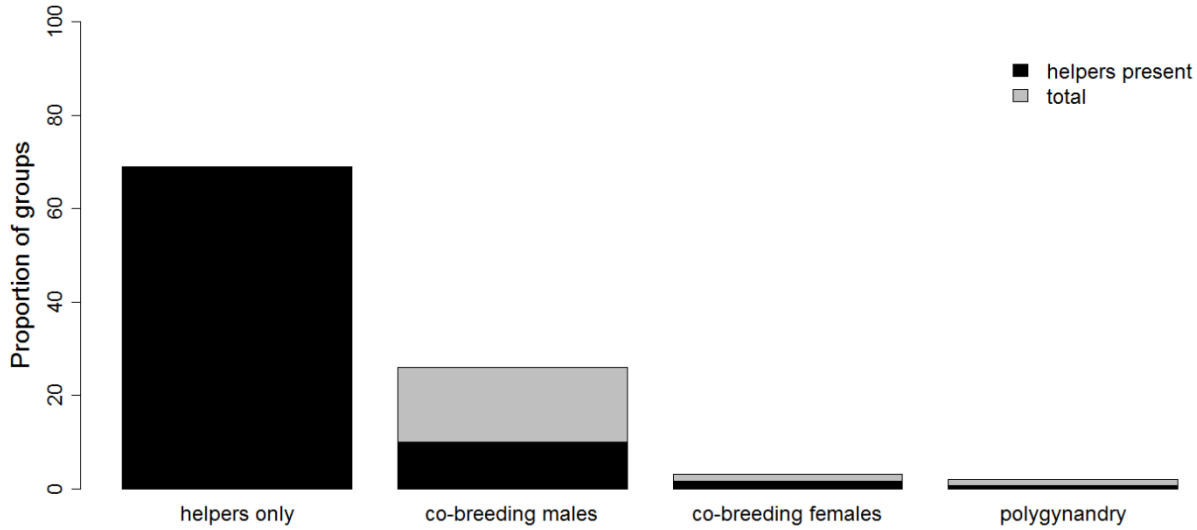

Figure 2. Social composition of brown-headed nuthatch groups. Proportion of groups of each social composition. Group compositions may change between years so each group is included for each year (2013-2014) (group-years).

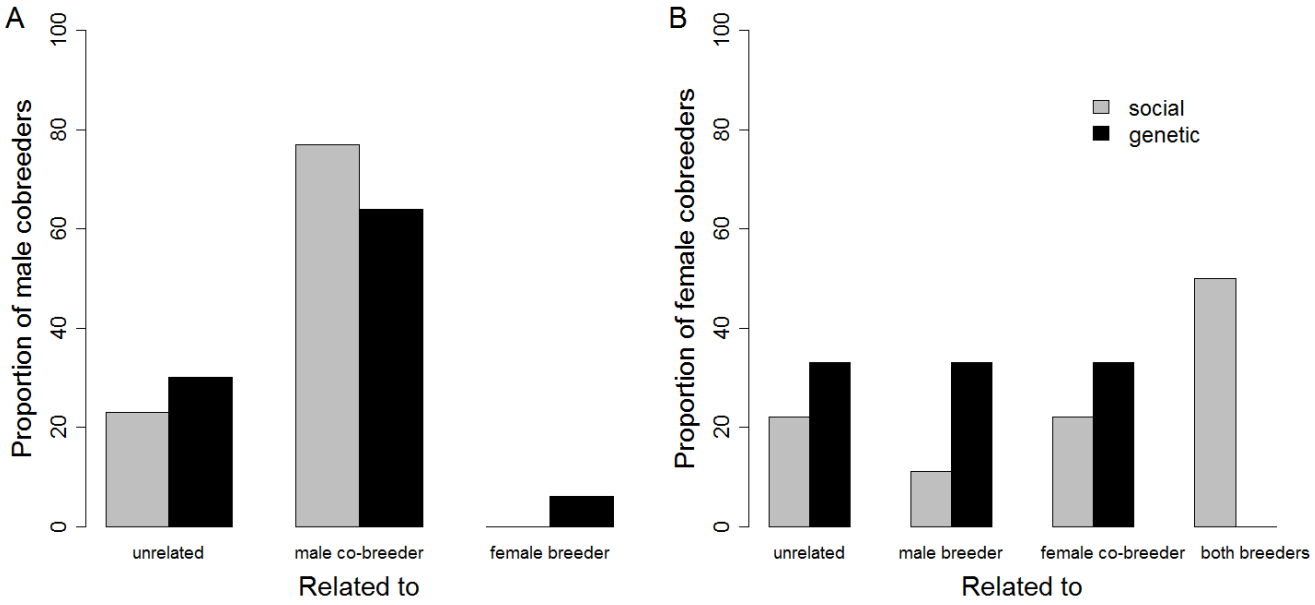

Figure 3. Social and genetic relationships between male and female co-breeders and their breeding partners. (**A**) Relationships (social and genetic) between male co-breeders and their breeding partners. (**B**) Relationships (social and genetic) between female co-breeders and the main breeder male in the group.

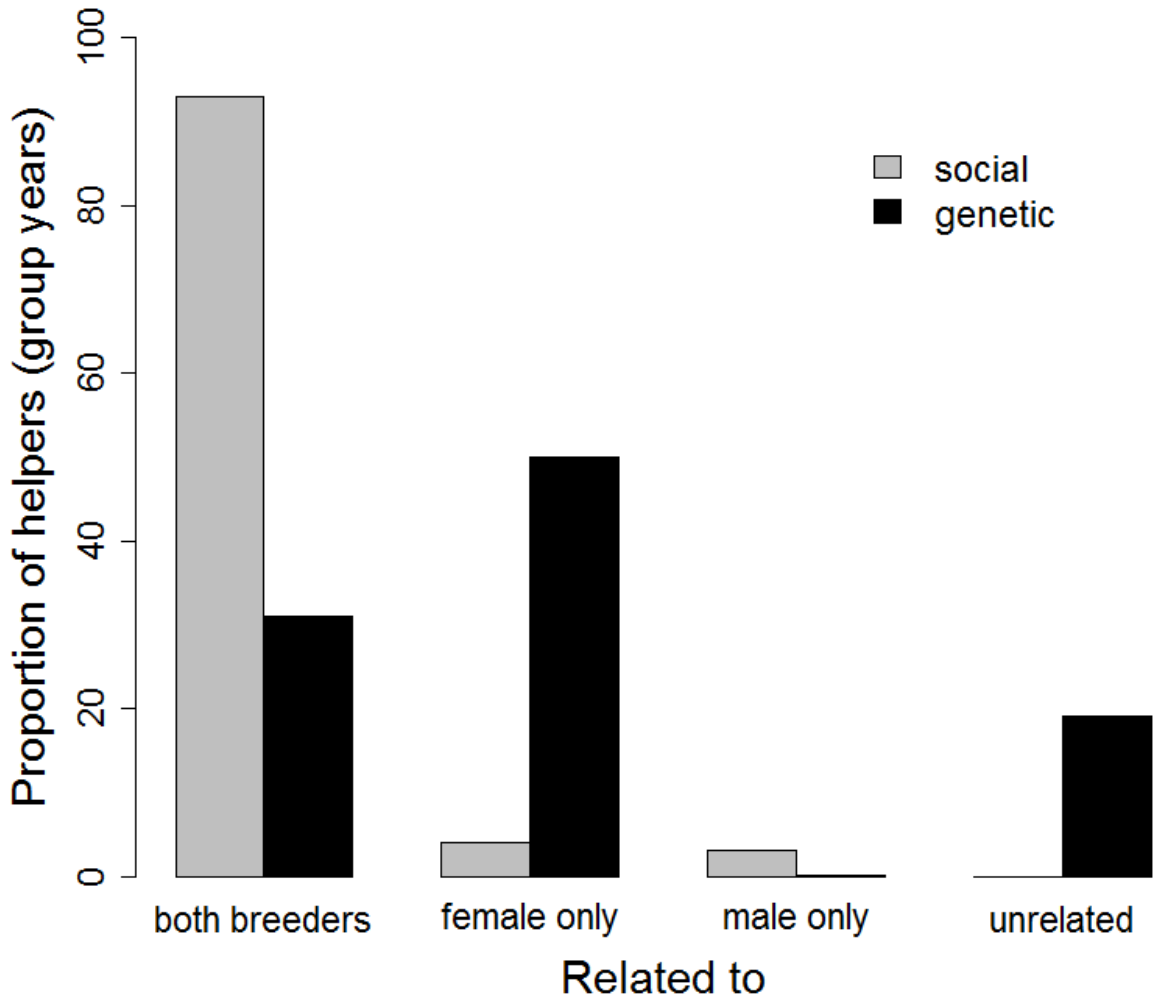

Figure 4. Social and genetic relationships between breeders and male helpers within breeding groups. Data treat group-years as independent units. Helpers can be related socially and genetically to the breeding pair. Being socially related (i.e. raised in their nest) did not guarantee genetic relatedness between social helpers and breeders, particularly between offspring and the breeding male(s).

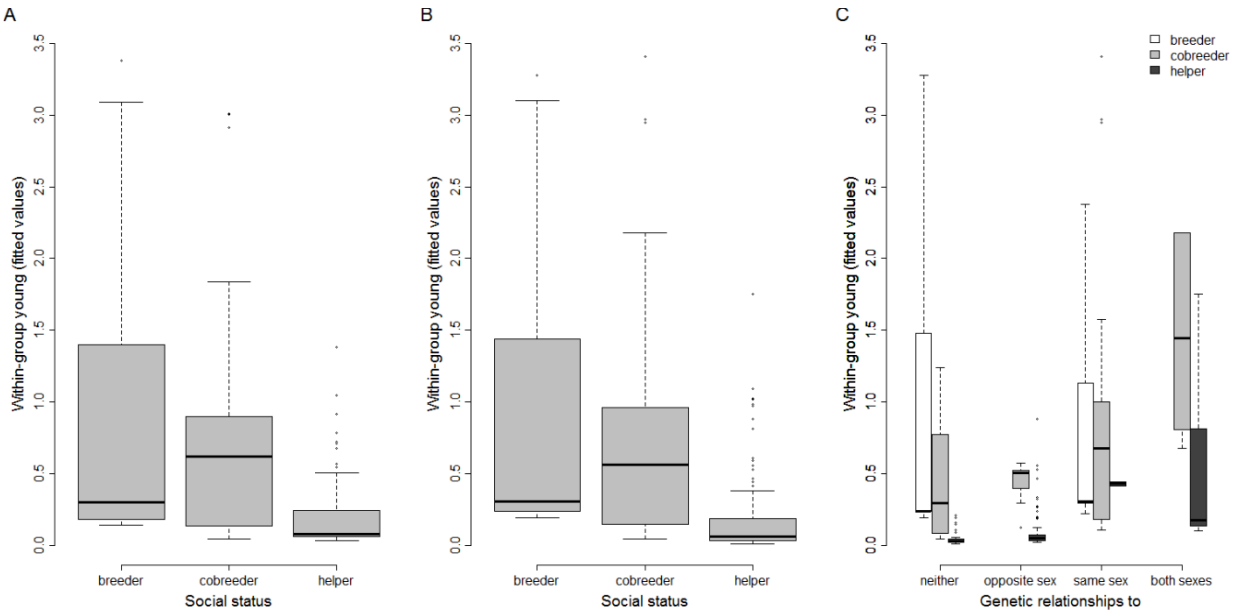

Figure 5. Effect of male social status and within-group relationships on number of young sired within the group. (**A**) Effect of socially inferred status on within-group young sired by males. Plotted values were from best supported model using social pedigree to infer relationships. Individuals may have a relationship to both the breeding male and female (both sexes), to only females within the group (opposite sex), to only males within the group (same sex), or to no group members (neither). (**B**) Effect of genetically inferred social status on within-group young sired by males. Plotted values were from best supported model calculating relatedness using genetic allele sharing to infer relationships between individuals. (**C**) Effect of the interaction between socially inferred status and genetic relationships between group members on the number of young sired by males. Relationships calculated as in **B**.

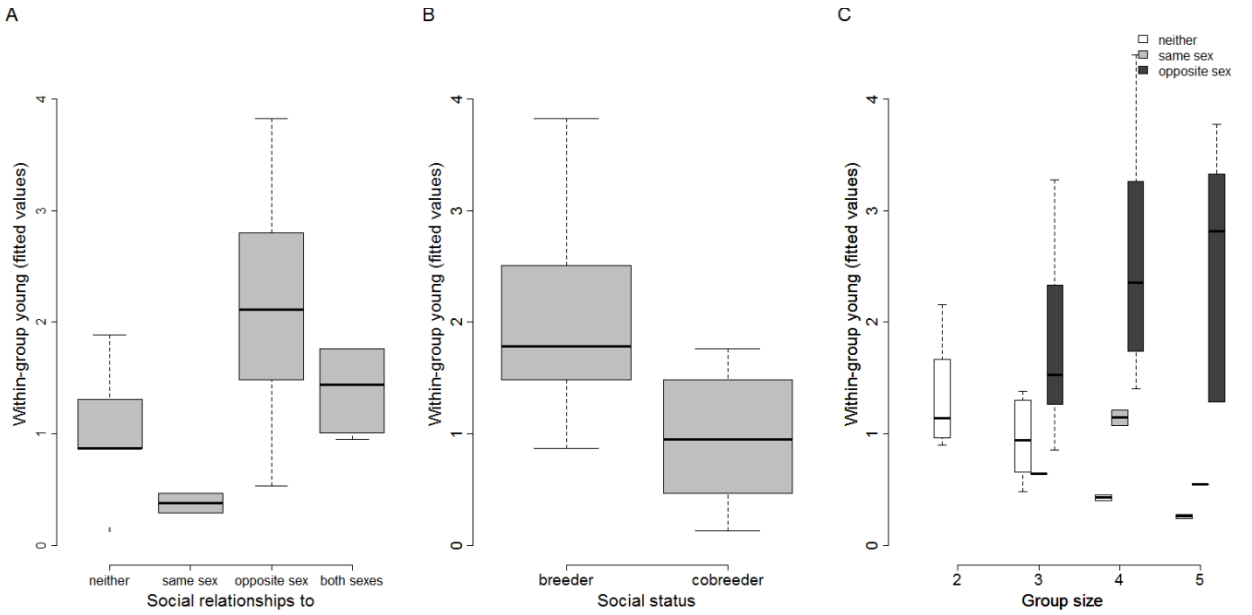

Figure 6. Effect of female social status and relationships to group members on within group reproductive success. (**A**) Effect of socially inferred relationship to other group members on number of within group young produced by females. Plotted values used relationships based on social pedigrees. Individuals may be related at the 0.5 level to both the breeding male and female (both sexes), to only females within the group (same sex), to only males within the group (opposite sex), or to no group members (neither). (**B**) Effect of social status on within-group young produced by females. Plotted values were from the best supported model using relationships based on shared alleles ($r_{xy} \geq 0.25$). (**C**) The interaction between group size and genetic relationship based on shared alleles ($r_{xy} \geq 0.25$) between group members on the number of young produced by females. Group relationship terms for B and C were defined as in **A**, except with genetic allele sharing rather than social pedigree information.

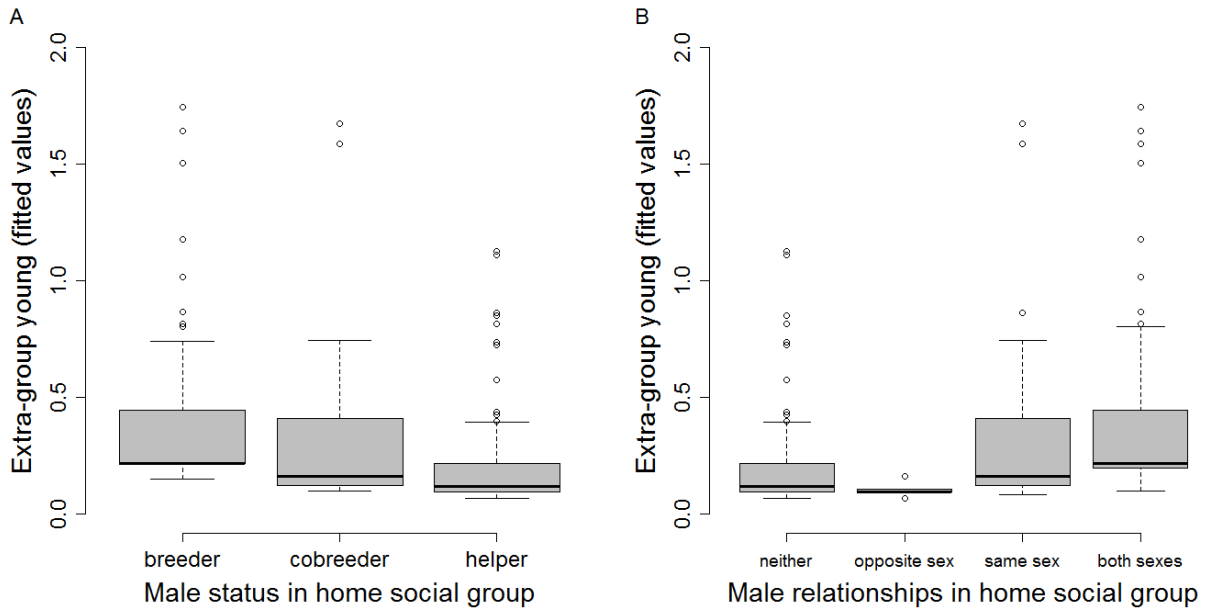

Figure 7. Impact of social status and relationships to breeders on male extra-group reproduction. (**A**) Effect of male social status on his extra-group paternity. Values plotted on the y axis were the fitted values from the most supported model (Poisson error distribution). (**B**) Effect of male relationships to breeders on his ability to sire extra-group young. Values plotted on the y axis were the fitted values from the most supported model (Poisson error distribution). Males may be related to both the male breeder and the female breeder (both sexes), to only the breeding female (opposite sex), to only the breeding male (same sex) or to neither the breeding male nor the breeding female (neither).